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BULLETIN
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A SEARCH FOR TROPHIC INFLUENCE OF THE SYMPATHETIC NERVOUS SYSTEM ON THE ADULT MAMMALIAN SKELETAL MUSCLE FIBER[1]

S. S. TOWER

From the Anatomical Laboratory, Johns Hopkins University

Skeletal muscle, considered as an organ, is sympathetically innervated. Anatomically, such innervation is certainly exhibited by the blood vessels. Whether or not the muscle fibers also support nerve endings of sympathetic derivation is a matter of controversy. In a paper now in press (1931) the author has supported the negative side. But irrespective of the anatomical distribution of the sympathetic nerves within the muscles, a problem exists as to the influence exerted by these on the fiber of skeletal muscle. This problem has presented several phases. The question of a tonic control has been decided. Following Hunter's (1925) positive findings in birds, fatigability was extensively investigated, by the author (1926) among others, and with negative results for the animal functioning as a whole. But experiments on isolated muscles have brought out, on the one hand, an exacerbating effect following sympathectomy (Kuntz and Kerper, 1924), and on the other, a mitigating effect of sympathetic stimulation on fatigue (Orbeli: see Gantt, 1929; Baetjer, 1930; and others).

Along another line are the attempts to determine whether the sympathetic nervous system influences the form of the striated muscle fiber in any way. With the exception of a report by Hartman, Evans, and others (1928) that visible changes took place during stimulation of sympathetic nerves—work which needs confirmation—the studies are all of the effects of sympathetic denervation, of longer or shorter duration. An array of mammals has been worked on,—rat, rabbit, goat, cat, dog, man. Hunter (1925) published the first positive finding; he described increasing numbers of very small fibers in the muscles of the goat's hind-leg and overgrowth of connective tissue, months after a

[1] Submitted for publication November 1, 1930.

sympathectomy. Kerper (1928) found more comprehensive changes in the case of cats and dogs, general reduction of fiber diameter and sarcomere length and nuclear proliferation. Gaissinsky and Lewantowsky (1929) ascribed atrophy extending to loss of longitudinal and cross striation and hyalinization, to a sympathectomy done four months previously. And Kuré and his collaborators (1925) reported sympathetic denervation as having a profoundly deleterious effect, strongly suggesting the picture of progressive muscular dystrophy in man, on the muscle fibers of one dog which survived operation ten months but not in animals which survived three, six and fifteen months. On the other hand, other workers, Floresco (1903), Takahashi (1922), Feldberg (1926) and Nevin (1930), were unable to make any morphological distinction between sympathetically denervated and normal muscle. However, only Takahashi and Nevin examined the limb muscles, and that somewhat cursorily. Similarly for man, Kuré and others (1926) described extensive and profound atrophic changes as developing in the muscles of the face, neck, arms and back following cervical sympathectomy. And yet Jonnesco and Floresco (1902), who studied a larger series of patients over a longer period of time after the same operation, found none.

For man, determination of the importance of the sympathetic innervation in maintaining trophic conditions in muscle must wait on opportunity in clinical medicine. But for the more common laboratory mammals, the author had accumulated a quantity of material suitable for the investigation of the problem.

MATERIAL: METHODS

Two cats, five dogs and two goats subjected to sympathetic denervation of one fore-limb, yielded the material for study. The cats and dogs were young adults; the goats were half-grown.

Except on one goat, the operation performed was always ganglionectomy. Under ether anaesthesia, through an incision parallel to the caudal border of the scapula, the stellate ganglion was exposed on one side in the vertebral end of the first intercostal space, and excised. The inferior and superior cervical ganglia and the cervical trunk were also removed through an incision the length of the neck in one cat (S V c) and one goat (G. III), and in the cat, the thoracic

sympathetic trunk down to the ganglion in the fourth interspace as well. In the remaining goat (G. V) the primary cords of the brachial plexus were exposed and the gray rami for C_6, C_7, C_8 and T_1, were cut as they joined the spinal nerves, according to the technique prescribed by Royle (1924). Autopsy confirmed the success of this operation, as well as the completeness of the ablations of sympathetic ganglia.

The animals were allowed to survive for a time after operation while the use of the limbs was being studied (Tower, 1926 and Tower and Hines, 1929). For the goats, this period was 8 weeks; for the cats, 4 months and 1 year; for the dogs, 28, 243, 254, 310 days and 2 years. The animals were then killed with ether. The cat and goat material was fixed by injection into the aorta, after the vascular system had been perfused with several liters of physiological salt solution. Specimens of dog muscle about 20 x 10 x 5 mm. were fixed by immersion. The fixative used was 20 per cent formalin, neutralized by saturation with magnesium carbonate.

After six months or more in the fixative, portions of the muscle were removed and stained according to a modified Bielschowsky silver impregnation technique, toned with gold and counterstained with alum carmine. Fuller details of the staining may be found in the report on the nerve endings in the material (Tower, 1931). This process of fixation and staining entailed shrinkage of the specimen to about one-half of its original volume. As far as possible, homologous specimens were removed from the three species of animal; in all three, from the junction of the proximal and middle thirds of the bellies of an extensor and flexor of the forearm (dog and cat: extensor digitorum communis and flexor digitorum profundus; in the goat: extensor digiti tertii proprius and flexor digitorum sublimis). In the goat and cat, material was also taken from the middle thirds of the bellies of the biceps and triceps (long head) and interosseous muscles *in toto*. Serial longitudinal (15μ) and cross (10μ) sections were made of each of these specimens except of the interossei; of these, longitudinal sections only. For every specimen from the side of operation, the corresponding normal was also removed and prepared.

Because of the long survival of most of these animals the possibility of nerve regeneration had to be considered, although removal of the post-ganglionic cell bodies from all except one goat rendered this un-

likely. At autopsy, regeneration of post-ganglionic fibers in this one goat had proceeded to the formation of nerve strands along the border of the stellate ganglion, but these did not reach the brachial plexus. Where sympathetic ganglia had been removed, there was evidence of preganglionic regeneration, a very short outgrowth even after one year in the cats, but in the dogs strands, many centimeters long, which wandered aimlessly beneath the pleura and in the loose tissues of the root of the neck. In all the animals in which the cervical ganglia remained, given sufficient time—six months or more—the cervical sympathetic had recovered at least partial control of the ocular functions. In not one of the animals, however, was there any evidence, such as vasomotor or sudomotor activity, to indicate that at the time of death sympathetic nerve fibers were present in the forelimb on the side of operation.

OBSERVATIONS

General morphological characteristics. A general survey of all the material soon revealed that the effect of sympathectomy on the morphology of the skeletal muscle fiber was, at most, very subtle. Allowing a certain variability to the Bielschowsky technique, the intensity and distribution of the stain were alike on the two sides. Considered more minutely, the cross striations stained equally sharply in both normal and operated specimens, and this also held good for the longitudinal striations. There was no fracturing along either of these planes or disarray of Krause's or Heidenhain's membranes, such as characterizes the earlier stages of atrophy after interruption of somatic motor innervation. The sarcoplasm gave no evidence of being increased or diminished; of being more granular or less granular, in the operated material. The nuclei were of the same staining intensity on both sides and showed no increase in size or number such as, again, characterizes the changes following somatic motor lesions. And lastly, the connective tissue was not obviously greater in amount on one side than on the other. The operated and normal preparations, whether from cat, dog or goat, were indeed quite indistinguishable by morphological characteristics. Plates I and II, showing, on the left, cross section of fascicles from normal muscles, and, on the right, from sympathetically denervated muscles, fail to disclose any evidence of the lesion.

Diameter of the muscle fiber. No difference in size was apparent between the muscle fibers of operated and normal preparations, nor was any inequality in the total muscle bulk demonstrable. But fiber size is a quality which lends itself to measurement and statistical treatment, as matters of intensity of staining and sharpness of striation do not. Therefore, with the aid of a mechanical stage and micrometer eye-piece, the diameters of one hundred fibers from each of the operated and normal specimens were measured and the limits and the average determined. The scale of the micrometer eye-piece was fixed in position, and the fibers brought under it at random. Ten fibers were measured in every tenth of one hundred cross sections. In this way an average was obtained of the diameter of the fibers occurring in one millimeter's length of specimens from the same region of sympathetically denervated and normal muscle. In all, the diameters of 5,200 fibers were measured. The results are presented in Table I.

The goat's muscle showed a remarkable agreement in average muscle fiber diameter, 15μ for both operated and normal specimens. The time that had elapsed after operation was, however, short,—only eight weeks. For the material derived from the cats which had survived longer, there was a final average discrepancy of 2μ in favor of the normal. But in the dogs which had survived even longer—the mean post-operative life being 384 days—this difference was reversed; the muscle fibers of sympathetically denervated muscle averaged a somewhat larger diameter than the normal, 37 as against 40μ. It is clear, therefore, that in so far as size may reflect the trophic condition of the fiber of skeletal muscle, this is not affected by sympathectomy even after many months' duration.

Types of fiber constituting the muscle. The manner of fixing the cat and goat material, by intra-aortic injection before the limb was skinned, precluded comparison of the gross color of the muscles on the operated and of those on the normal side. Such observations were made only on the dogs. In these it was never possible to see that the sympathetically denervated muscle differed from the normal in color or shade in the slightest degree. But as Denny-Brown (1929) has emphasized, gross muscle color and fiber constitution do not go entirely hand in hand. The material was then surveyed for the discrimination of fiber types, and an attempt made to determine whether

TABLE 1

Showing average diameter of muscle fibers and range of variation

NUMBER OF ANIMAL	SURVIVAL TIME	MUSCLE	NORMAL		OPERATED	
			Average diameter	Range of diameter	Average diameter	Range of diameter
Dogs						
	days					
DI 6	730	Flexor	35μ	17–60μ	38μ	23–62μ
		Extensor	37	23–56	40	26–56
DII 12	310	Flexor	41	27–63	45	30–60
		Extensor	39	26–53	43	33–56
DII 18	254	Flexor	35	27–60	39	23–53
		Extensor	42	23–68	43	31–60
DII 17	243	Flexor	35	20–53	36	20–53
		Extensor	35	20–60	35	10–60
Average	384		37μ		40μ	
DII 21	28	Flexor	35μ	20–56μ	35μ	20–56μ
		Extensor	34	20–53	31	17–50
Average	28		34μ		33μ	
Cats						
SV b	112	Flexor	28μ	17–37μ	26μ	18–39μ
		Extensor	30	17–47	27	17–45
		Biceps	27	18–48	27	20–47
		Triceps	35	17–33	29	6–53
SV c	364	Flexor	30	18–46	27	14–45
		Extensor	29	17–48	25	10–49
		Biceps	27	10–54	26	10–48
		Triceps	27	10–53	28	10–59
Average	238		29μ		27μ	
Goats						
G III	56	Flexor	15μ	6–27μ	16μ	7–24μ
		Extensor	13	7–24	12	6–24
		Biceps	15	9–30	15	6–43
		Triceps	16	6–29	15	6–24
G V	56	Flexor	16	7–27	16	7–29
		Extensor	14	6–27	13	6–24
		Biceps	15	6–27	17	6–27
		Triceps	14	4–26	16	6–30
Average	56		15μ		15μ	

the composition of the muscle by fibers of diverse character had been altered in proportion or clearness by sympathetic denervation of long standing.

Dog. All the muscles of the forelimb of the dog are red on gross examination, with little difference in depth of color from one to another. In the study of the sections an effort was made to determine if the muscle which, grossly, was homogeneous was in reality mixed. But neither on the basis of fiber size nor of staining reaction could different kinds of muscle fibers be distinguished (figs. 13–16). At most, some fibers presented a more closely packed, more even cross section than others in which Cohnheim's fields stood out more distinctly (fig. 16). But all were characteristically large, rich in sarcoplasm and deeply staining.

Watch was kept for small fibers of the type described by Hunter (1925) as present in increased numbers in sympathetically denervated goat muscle. But extraordinarily few small fibers were encountered either in the normal or in the operated material, fewer even than were anticipated as the ends of fibers. Preparations of fresh normal muscle dissociated in 20 per cent nitric acid showed, however, that the muscle fibers in the forelimb of cats and dogs terminate quite abruptly in a tendon filament. Up to within a distance of this point, generally little more than the diameter of the fiber, there is only slight tapering.

In three of the Bielschowsky preparations, all from the normal side, as it happened, fibers of unusual size, 60μ. or more, were occasionally encountered. These fibers presented a peculiar glassy appearance and the shape of the surrounding muscle fibers was distorted so as to give them a roundness. What these fibers represent, whether fixation artefact or degenerating fibers in an otherwise normal muscle, cannot be hazarded. In appearance, however, they were most suggestive of the hypertrophic fibers described by Kuré *et al.* (1925) as appearing in dog's muscle after either sympathectomy or dorsal root section, and also in progressive muscular dystrophy in man. Such large round fibers were also found twice in material from the operated side of one cat (S V b), and several times in normal goat material.

Cat. The cat material afforded an opportunity to attempt correlation between gross color of the muscle and fiber composition. Grossly, the flexor digitorum profundus muscle, is red, whereas the

other three muscles examined are pale, the triceps (long head) the palest. Yet examination of the stained cross sections showed all four of these muscles in one cat (S V b) to be composed of fibers classifiable into two groups (Plate I), while in the second cat (S V c) dissimilar fibers could be clearly distinguished only in the biceps and triceps muscles, and in the latter, there seemed to be three types, rather than two. The preponderating fiber, in the ration of about 10 or 12 to 1, (varying sometimes between 2 to 1 and 20 to 1 in different parts of the same section) was on the whole slightly the larger, averaging around 30μ., and presented the more loosely packed and granular appearance (Plate I). In S V b it was the more deeply stained component; in S V c the less. The rarer fiber averaged somewhat smaller, 20μ., was characteristically more homogeneous in cross section and without conspicuous granules. These smaller fibers were scattered among the larger ones, and were not collected in groups; in some of the fascicles none were seen. Also, discrimination was less easy in cross sections of fascicles through the region of the motor nerve endings. Considering the variability of the staining reaction and the inconstancy of the occurrence of fiber types in the two cats, any attempt to correlate fiber composition of the muscle with gross color seems unwarranted. And even more so, in view of the irregular distribution of the types, when present, throughout a small cross section, would be any statement as to the effect of sympathectomy on the constitution of muscle by fibers of peculiar character. It must be said, moreover, that between the extremes considered as types, intermediate gradations of size and appearance could always be found.

Goat. In examining the goat's muscle preparations certain interesting features appeared. For each muscle examined the connective tissue bulked larger and the fibers were smaller than in the homologous muscle of the cat or dog (the goats were half grown). The fiber staining in the flexor digitorum sublimis (figs. 9 and 10) and extensor digiti tertii proprius was remarkably constant; uniformly, the fibers were of a pale staining, somewhat loosely packed variety. Neither on the basis of staining reaction nor of fiber size were diverse types suggested. Curves of percentage distribution of fiber size were quite smooth, presenting one peak at 12μ. for both normal and operated material. In the biceps and triceps muscle, on the contrary, light and dark

staining of the fibers was a conspicuous feature (figs. 11 and 12). Among more numerous dark fibers, the light staining, loosely packed fibers stood out prominently. The light fibers were perhaps a trifle larger on the whole than the dark, sufficiently to prolong the curves of size distribution in the range of the larger sizes but not to create a second peak in this curve which reached its maximum at 10μ. The numerical ratio of dark to light fibers was approximately 3 to 2. Small fibers were not found in excessive numbers in the operated material, as described by Hunter (1925). Indeed, the dark fibers, which were very slightly the smaller, were less numerous on the side of operation in some of the material.

The types of fiber discriminated in muscle are commonly described as "large pale fiber" and "small granular fiber." Denny-Brown (1929) has, however, pointed out that conditions are not so simple as this nomenclature would indicate. Thus for the cat, considering fiber size and homogeneity of the cut surface, a large granular fiber, rich in sarcoplasm, and a small pale non-granular homogeneous fiber were distinguished. Whereas in the goat, on the basis of staining reaction the fibers were described as light and dark. The terms "large pale" and "small granular" were in no case applicable. What such characteristics may signify for muscle function, difference in contraction rate, in the ability to store specific lipoidal or protein products, does not lie within the province of this paper to consider. Yet the problem is of prime importance. For not until the conditions are known which gave rise to clear-cut fiber types in one cat and not in a second; not until the distribution of such diverse fibers within the muscle shall be better understood, will it be possible to evaluate small quantitative changes, the possible result of lesions of the sympathetic. None the less, from the study of the cat and goat material it is possible to say, that sympathetic denervation even of many months' duration resulted in no alteration of the fiber constitution of the muscle which was greater than the exceedingly great variability of the same in the normal control material.

DISCUSSION

The literature contains a great deal of evidence purporting to show alterations in the morphology of the fiber of skeletal muscle of one

sort or other following sympathectomy. The literature also contains an almost equal number of reports by workers who could not detect any such effects. All these publications are characterized in general by a lack of regard for quantity production of evidence on a point involving considerable normal variability, and by a dearth of accurate measurement. The present paper has attempted to fill both these defects. A large quantity of material from three species of animals was carefully studied, normal and sympathetically denervated specimens being always examined side by side. Each morphological characteristic—staining reaction, striation, nuclei, connective tissue—was examined in turn, and the composition of the muscles by fiber types surveyed. And finally, the diameter of 5,200 fibers—half normal, half operated,—was measured. In the bulk of these data lies the only claim of the present report to greater validity than its predecessors. And from none of the morphological characteristics examined could any distinction be made which would discriminate the sympathetically denervated fiber of skeletal muscle from its normal fellow. The conclusion seems justified, therefore, that the local sympathetic innervation of muscle exerts no trophic control on the fiber of skeletal muscle which achieves morphological expression.

SUMMARY

A series of cats, dogs and goats were subjected to unilateral sympathetic denervation of one month to two years' duration. Specimens of muscle from the operated and normal fore-limbs were prepared according to a modified Bielschowsky technique, and carefully studied.

Inspection of this material showed: (1) staining reaction of equal intensity in operated and in normal material; (2) longitudinal and cross striations equally sharply stained on both sides and no tendency to fracture along either of these planes; (3) no increase in size or number of nuclei; (4) no increase in connective tissue.

Measurement of the diameter of 5,200 muscle fibers yielded an average diameter of 2,600 muscle fibers from sympathetically denervated muscle exactly equal to the average diameter of the same number of fibers from normal muscle.

Study of the constitution of muscle by fiber types failed to demonstrate any effect of sympathetic denervation in excess of a very great normal variability in this characteristic.

The conclusion is reached that the local sympathetic innervation exerts no trophic control on the form of the extrafusal muscle fiber of skeletal muscle.

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PLATE I

Untouched contact prints of photomicrographs made with 4 mm. apochromat. objective, × 10 compens. ocular.

Cat S V b; right stellate ganglion removed four months before fixation. Cross sections of muscle fascicles: figures at left from normal specimens; at right, from corresponding sympathetically denervated specimen.

FIGS. 1 AND 2. Flexor digitorum profundus.

FIGS. 3 AND 4. Extensor digitorum communis.

FIGS. 5 AND 6. Biceps brachii.

FIGS. 7 AND 8. Triceps brachii.

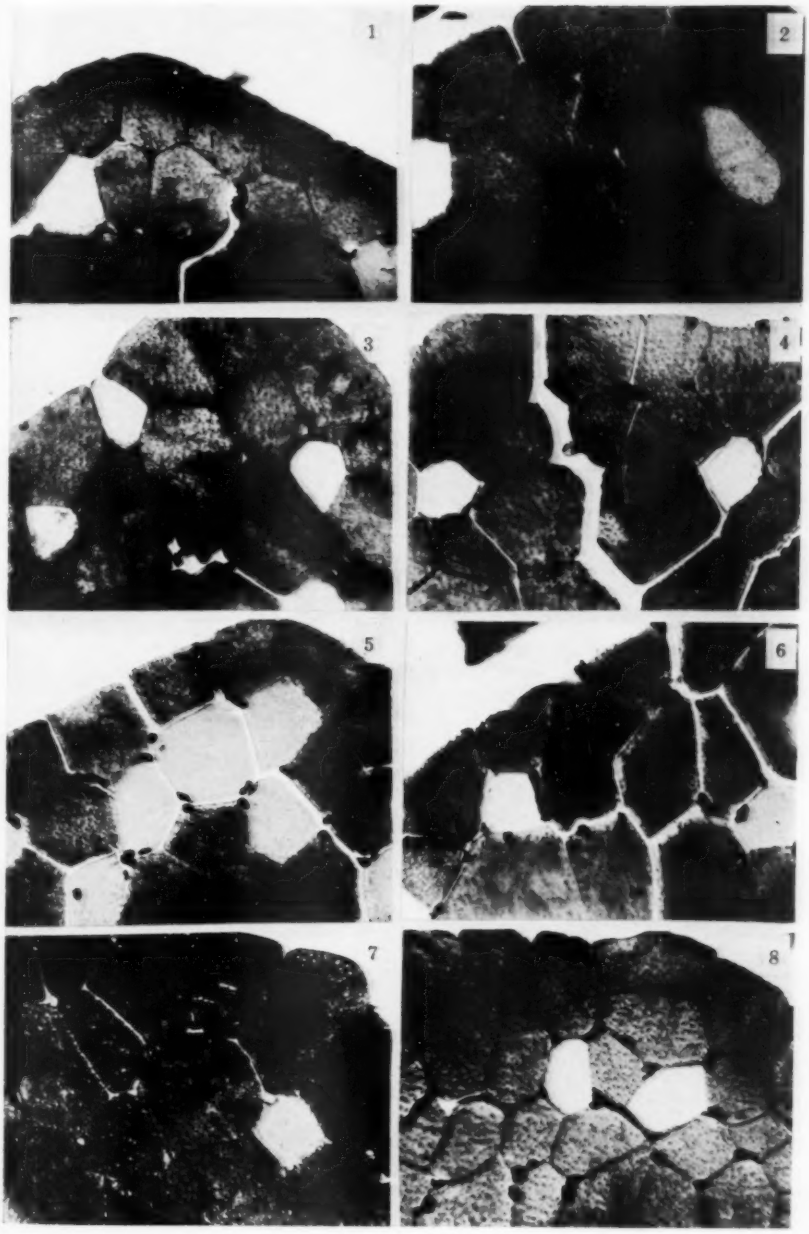
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PLATE II

Untouched contact prints of photomicrographs made with 4 mm. apochromat. objective, × 10 compens. ocular.

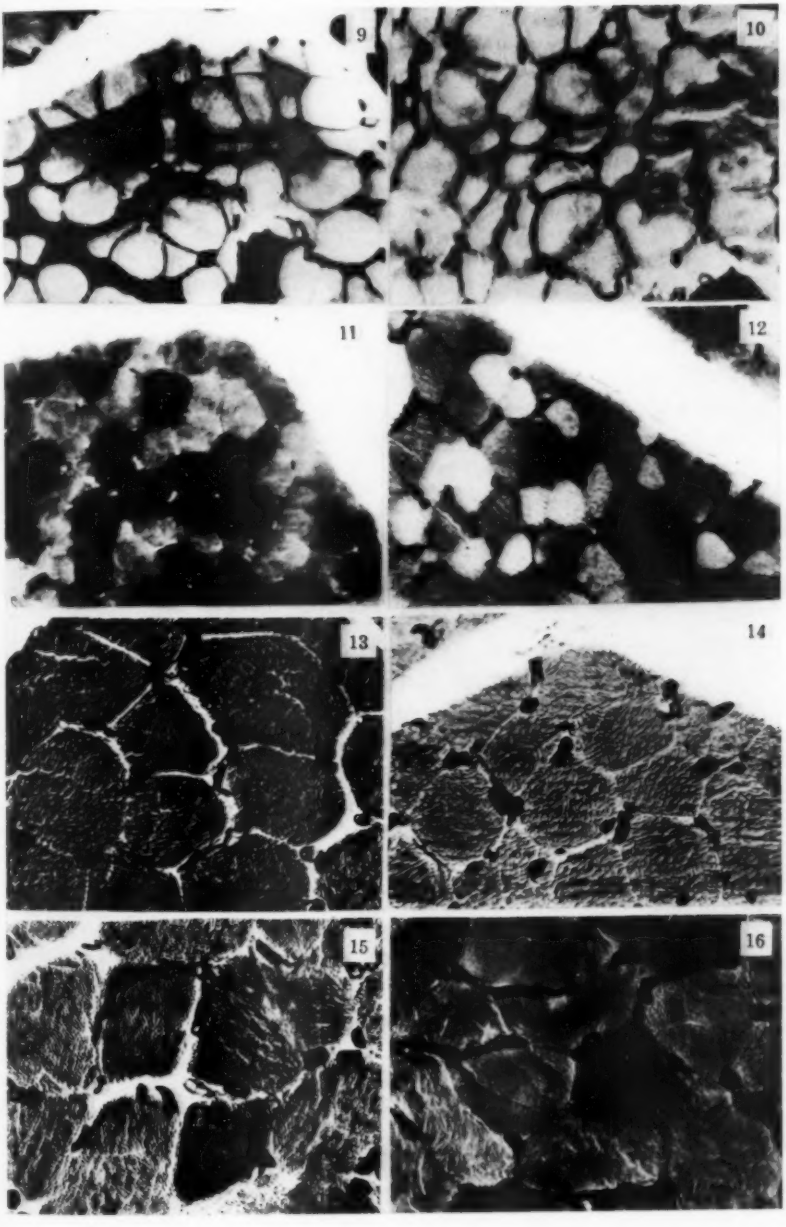
Cross sections of muscle fascicles: figures at left from normal specimens; at right, from corresponding sympathetically denervated specimens.

FIGS. 9 AND 10. Goat III; right stellate ganglion and cervical sympathetic chain removed 56 days before fixation; flexor digitorum sublimis.

FIGS. 11 AND 12. Goat V; left grey rami communicantes C.6 – T.1 cut 56 days before fixation; triceps brachii.

FIGS. 13 AND 14. Dog 17; right stellate ganglion removed 243 days before fixation; flexor digitorum profundus.

FIGS. 15 AND 16. Same; extensor digitorum communis.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THE IMPORTANCE OF DIET IN PREVENTING ACUTE YELLOW ATROPHY DURING ARSPHENAMINE TREATMENT[1]

E. B. CRAVEN, JR., M.D.

From the Department of Pathology, The Johns Hopkins University, Baltimore

Since the publication in 1919 of Davis and Whipple (1) concerning the influence of fasting and various diets on the liver injury effected by chloroform anaesthesia, in which the authors concluded that diets rich in carbohydrates exert a marked protective action against the liver injury caused by chloroform, whereas diets rich in fat and protein afford a maximum susceptibility to such injury, it has been assumed by various clinicians that the foregoing conclusions might be applied to the toxic effects of arsphenamine as well.

Thus, it is a well established practice today in certain clinics to put patients suffering from arsphenamine jaundice on a high carbohydrate diet. Westrope (2), in 1916, advised that a milk diet be given 24 hours prior to the administration of arsphenamine. Bailey and MacKay (3), in 1920, in a study of toxic jaundice in patients under arsphenamine treatment, state that: "It would seem advisable in the treatment of Syphilis to institute a rich carbohydrate diet very low in fat and protein." These authors cite the experiments of Davis and Whipple (1) on chloroform poisoning, and, arguing by analogy, conclude that the same principles are applicable in arsphenamine poisoning.

To us, it did not seem necessarily to follow that the efficacy of a high carbohydrate diet in preventing liver injury from chloroform poisoning would hold for a drug so chemically dissimilar as arsphenamine, even though the type of liver injury, namely, central necrosis, is the same in both.

Accordingly, a set of experiments was devised to test the protective

[1] Submitted for publication January 5, 1931.

action of the three principal diets, high carbohydrate, high fat, and high protein, against the liver injury produced by arsphenamine.

Hooper, Kolls, and Wright (4), in 1921, stated that rats maintained on a diet of rolled oats, white bread and milk are much more resistant to the toxic effects of arsphenamine than are rats on a lean meat diet, but that this resistance was markedly diminished by feeding 40 per cent saccharose solution and by fasting. The chief pathological lesion produced in their rats was necrosis of kidney epithelium. Out of 600 rats on various diets only 10 exhibited central liver necrosis.

In experiments on dogs, these authors were apparently not particularly concerned with the effect of diet. In a series of 23 dogs which were given disodium arsphenamine, monosodium arsphenamine, and various salts of mercury in combination with arsphenamine, only four developed jaundice with accompanying liver damage. Three out of the series of 23 dogs were maintained on a mixed diet consisting of 150 grams lean beef; 150 grams dog biscuit; 60 grams milk powder; and 280 cc. water daily. Of these three dogs, one (33 per cent) developed jaundice and central liver necrosis. The other 21 dogs were maintained on either a medium fat beef diet or lean beef alone. Of these, three (15 per cent) developed jaundice and central liver necrosis.

The mixed diet mentioned above cannot be considered a high carbohydrate diet. From the experiments quoted, the conclusion that the medium fat beef and lean beef diets predisposed to liver necrosis is hardly warranted.

EXPERIMENTS

Arsphenamine

A standard brand of arsphenamine was used throughout. The acid arsphenamine was dissolved in sterile distilled water, so that each 10 cc. of solution equalled 0.10 gram of arsphenamine. The arsphenamine was then precipitated by the addition of 15 per cent sodium hydroxide and enough excess sodium hydroxide added to redissolve the precipitate. With the solution perfectly clear, a single drop of NaOH was then added and the solution filtered. Enough solution was made up each time it was used so that an entire series of animals might be injected with the same lot.

Diets

1. The carbohydrate diet consisted of boiled rice liberally sprinkled with sugar and made into a paste by the addition of skim milk. The rice was rolled into balls and covered with cracker meal.

2. The fat diet consisted of either beef or pork fat, or both, with the addition of small quantities of lean beef. It was found necessary to add this lean meat because the dogs would not eat fat alone. In such a mixture the fat by weight always amounted to 70 to 75 per cent of the total.

3. The protein diet was made up of lean beef without any bones and with a negligible quantity of fat.

4. Water was supplied *ad libitum*.

In all these experiments, the dogs, after being weighed on admission, were kept on their respective diets for at least four days prior to the administration of arsphenamine. Their weights on the day of arsphenamine injection, have, without a single exception, been in excess of the admission weights.

Early orientation experiments convinced us that small doses of arsphenamine, such as the maximum therapeutic dose of 0.01 gram per kilo of body-weight would not produce jaundice even after several weekly injections. Accordingly, the dose was increased to 0.02 gram per kilo in order that the experiments might not be unduly prolonged.

Experiment I. Four dogs were used. Two were given the high carbohydrate diet and two the high fat diet. Four days later all four dogs were given 0.02 gram of alkalinized arsphenamine per kilo of body-weight. On the second day following, one of the carbohydrate dogs (3C) developed a plasma jaundice (Van den Bergh test). The sera of the other three dogs were negative for bilirubin. The jaundiced animal was killed with ether and one of the fat animals (dog 4F) was sacrificed as a control. Autopsy of the jaundiced dog showed all of the organs essentially normal excepting the liver. The liver was pale and slightly wrinkled. Over the surface were scattered numerous small dark red areas which were sunken slightly below the surface. The cut surface disclosed lobules packed with fat, with a translucent zone around the central spaces. Microscopic study showed a typical central liver necrosis (Fig. 1). The cells for an extensive area around the central veins were necrotic. There was a great deal of intracellular fat in all the liver cells. The other organs were normal.

Autopsy and microscopic study of the "fat" dog (4F) showed no liver necrosis (Fig. 2). There was a moderate amount of fat in the cells about the portal spaces. The other organs were normal.

One week after the first arsphenamine injection, the remaining two dogs were injected with the same quantity of arsphenamine as before. On the second day following the injection, the blood serum of "carbohydrate" dog (1C) contained bilirubin. The serum of dog 2F (fat diet) was perfectly clear and gave a negative Van den Bergh reaction. Both dogs were killed with ether. Grossly, the liver of "carbohydrate" dog (1C) was shrunken, with exceedingly numerous dark red

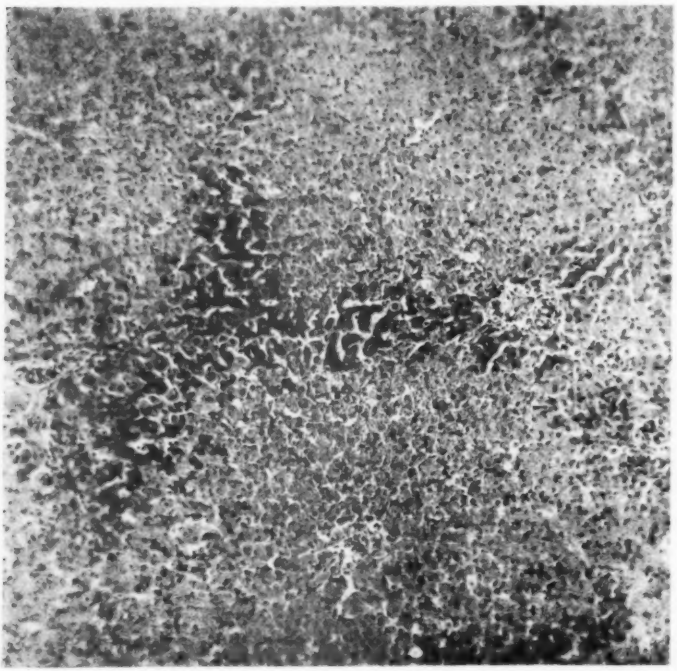

FIG. 1. DOG 3C. LOW-POWER MICROPHOTOGRAPH SHOWING EXTENSIVE CENTRAL LIVER NECROSIS IN "CARBOHYDRATE" ANIMAL AND PRESERVATION OF CELLS AROUND PORTAL SPACES

sunken areas scattered over the surface. The other organs appeared normal. Microscopic study showed a marked collapse of liver structure. The bile-ducts were brought very close together, so that in some low-power fields as many as twelve or fourteen could be counted. There was considerable fresh central necrosis. The impression here was that many of the necrotic cells had been removed, with a resulting collapse of liver structure. Around the portal spaces there was evidence of regeneration with sprouting bile-ducts.

Autopsy of the "fat" dog (2F) showed nothing abnormal. The liver was smooth and full and seemed perfectly normal. Microscopic study showed no necrosis.

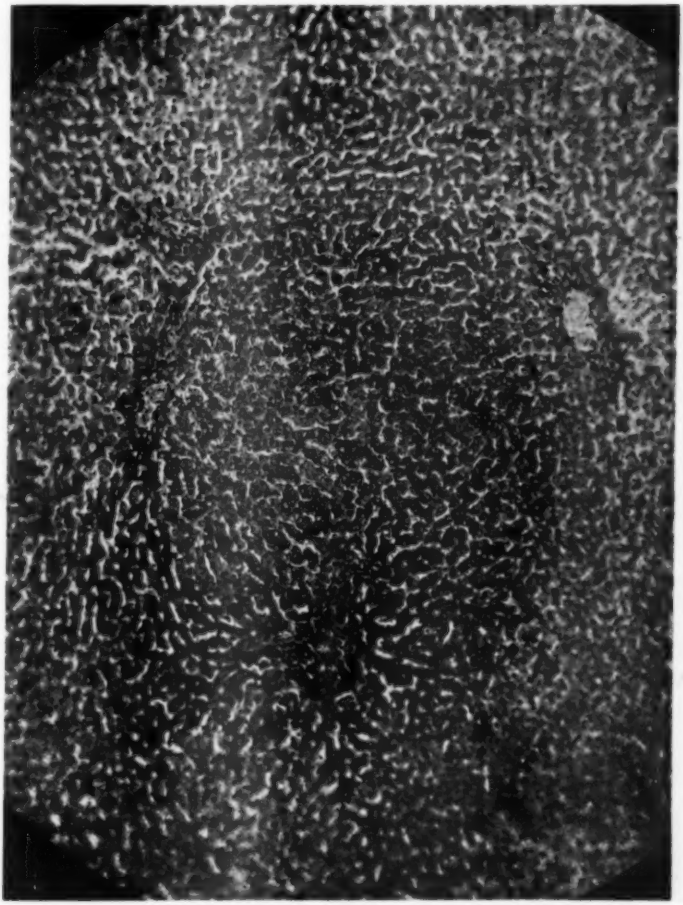

FIG. 2. DOG 4F. LOW-POWER MICROPHOTOGRAPH OF "FAT" ANIMAL. SHOWING ABSENCE OF CENTRAL NECROSIS

Experiment II. Nine dogs were used. Three were on carbohydrate diet, three on fat diet, and three on protein diet. Seven days after the beginning of the

diets each dog received arsphenamine to the amount of 0.02 gram per kilo of body-weight. Van den Bergh tests were carried out on the blood sera every other day with negative results. One week after the initial injection each dog again received the same quantity of arsphenamine as before. Van den Bergh tests were again negative. Accordingly, a third injection was given one week after the second injection. On the second day following, the sera of all three dogs on the carbo-

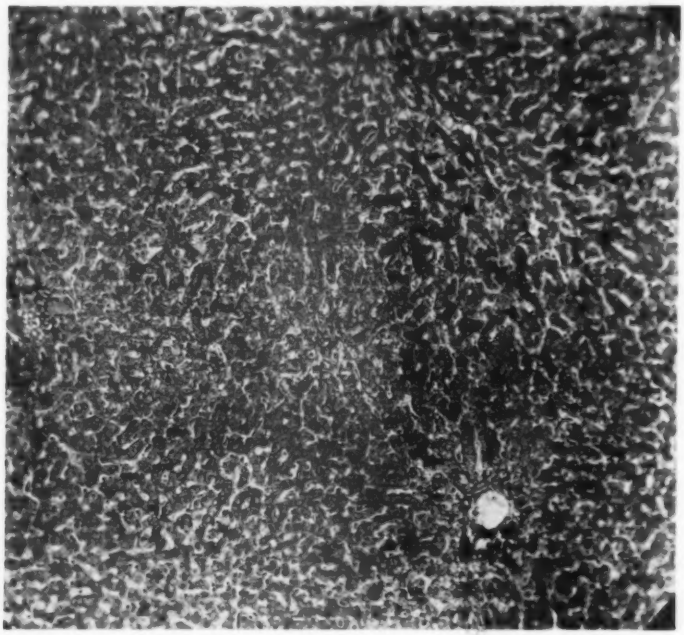

FIG. 3. DOG 14M. LOW-POWER MICROPHOTOGRAPH OF "PROTEIN" ANIMAL. SHOWING ABSENCE OF CENTRAL NECROSIS. MODERATE QUANTITY OF INTRACELLULAR FAT

hydrate diets contained bilirubin. Autopsy and microscopic study showed marked liver damage, with great collapse of liver structure, areas of necrosis and regeneration.

The serum of one of the three dogs of the fat diet (Dog 6F) also gave a faintly positive reaction for bilirubin. Microscopic study showed slight collapse of liver structure but no fresh necrosis.

The sera of the other two dogs on the fat diet and of the three dogs on the protein

diet were perfectly clear and gave a negative Van den Bergh reaction. Autopsy and microscopic study showed no liver necrosis (Fig. 3).

Experiment III. Nine dogs were used, three being placed on each diet. Five days after beginning his diet, each dog received arsphenamine to the amount of 0.03 gram per kilo of body-weight. On the second day following the injection "carbohydrate" dog (23C) became jaundiced. The sera of the remaining eight dogs were negative. Dog (23C) together with dog (15F) as a control were sacrificed. Gross and microscopic study showed extreme central liver necrosis in the "carbohydrate" animal and no necrosis in the fat animal.

One week after the first injection, arsphenamine was again administered to the remaining seven dogs in the same dosage. On the second day following this injection, the sera of the two remaining "carbohydrate" animals were markedly jaundiced. Autopsy and microscopic study showed extensive central liver necrosis in both animals. One of the two remaining dogs on the fat diet (dog 17F) also gave a faintly positive Van den Bergh reaction. Autopsy and microscopic study failed to disclose any liver necrosis.

The sera of the three dogs on the protein diet and that of the remaining "fat" dog were negative. Autopsy and microscopic study showed no necrosis in the liver of the "fat" animal, while in the livers of the three "protein" animals there were small areas of necrosis around some of the central veins. In quantity this necrosis was slight in comparison with that found in the liver of the three "carbohydrate" animals.

Experiment IV. Eight dogs were used—two on carbohydrate diet, two on fat diet, and four on protein. Eight days after beginning the diet each dog received intravenously arsphenamine to the amount of 0.03 gram per kilo of body-weight. On the second day following this injection the sera of both "carbohydrate" animals and one "fat" animal (dog 28F) became intensely jaundiced. Autopsy and microscopic study showed a most extensive central liver necrosis in all three animals.

The sera of the four dogs on the protein diet and of the remaining dog on the fat diet were negative for bilirubin. Autopsy and microscopic study showed, in the case of the "fat" animal, no liver damage. Of the four dogs on the protein diet, the livers of three showed quite definite necrosis around the central veins, not, however, nearly so extensive as that observed in the "carbohydrate" dogs. The liver of the fourth "protein" animal was essentially normal.

DISCUSSION

Reference to table I shows that, of a series of thirty dogs, ten each were placed on a high carbohydrate, a high fat, and a high protein diet. Arsphenamine was given to these dogs in exactly equal amounts based on the body-weight of the animals. Yet the extent of liver in-

jury suffered by these animals varies greatly. Of the ten "carbohydrate" animals there was extreme liver necrosis (necrosis of the entire lobule with the exception of a narrow zone around the portal spaces) in four, marked necrosis in five (necrosis of a third to half of the lobule) and moderate necrosis in one.

Of the ten "fat" dogs there was frank necrosis in only one. It seems probable that the injury here was due to starvation. This will be

TABLE I

"COH" DOGS		"FAT" DOGS		"PROTEIN" DOGS		"STARVATION" DOGS		
Jaundice	Necrosis	Jaundice	Necrosis	Jaundice	Necrosis	Jaundice	Necrosis	
+	Marked	0	0	−−	−−			0.02 gram per kilo, 2 doses
+*	Extreme	0	0	−−	−−			
+	Marked	0	0	0	0			0.02 gram per kilo, 3 doses
+	Moderate	0	0	0	0			
+	Marked	+0	Very slight	0	0			
+*	Extreme*	0	0	0	Slight			0.03 gram per kilo, 2 doses
+	Moderate	+0	0	0	Slight			
+	Marked	0	0	0	Slight			
+	Extreme	0	0	0	0			0.03 gram per kilo, 1 dose
+	Extreme	+	Extreme	0	Moderate			
−−	−−	−−	−−	0	Moderate			
−−	−−	−−	−−	0	Moderate			
				0	0	+	Extreme	0.03 gram per kilo, 1 dose
				0	0	+	Extreme	

* Jaundice after first dose.

discussed later. One other "fat" dog exhibited a doubtfully positive Van den Bergh. Microscopically, there was no fresh necrosis, but there appeared to be a slight collapse of the lobule as if liver cells had been killed and removed.

Of the ten "protein" animals, four appeared normal, three exhibited slight central necrosis, consisting of a narrow zone of necrotic cells about the central veins, and three showed a necrosis of one-tenth or less of the lobule, designated as moderate necrosis.

During the course of the experiments it was noted from time to time that an occasional dog would not eat the diet provided. When possible, such dogs were removed and others substituted. Dog (28F) represents an animal which ate well until two days before the arsphenamine was given and then refused his diet. Nevertheless, arsphenamine was injected. He promptly became jaundiced and at the autopsy showed extensive liver damage.

To test the hypothesis that starvation was the predisposing factor here, a small series of four dogs was used. Two of these animals were starved for forty-eight hours (water *ad lib.*). The other two were fed a meat diet. Arsphenamine was then given in a dosage of 0.03 gram per kilo of body-weight.

On the following day, both of the starvation animals were very sick. They had vomited repeatedly, were very weak and listless, and were jaundiced. All four animals were sacrificed. The two "starvation" animals showed extreme liver necrosis. The two "protein" dogs showed no liver damage.

The clinical observation has been made that patients receiving a "course" of arsphenamine will occasionally become jaundiced several weeks or months after the last injection. Various explanations have been given for this. It has been said that, in such a case, the delayed jaundice is due to other factors, perhaps infectious in nature. Others have hypothesized a cumulative effect of the arsphenamine.

In view of the marked predisposition to liver injury shown by the "carbohydrate" animals in the above experiments, it seemed possible that delayed jaundice following arsphenamine treatment might be due to a "carbohydrate debauch."

To test this hypothesis, it became desirable to keep a series of dogs on a meat-fat diet for several weeks and give them weekly injections of arsphenamine. The attempt was made to give them a quantity of arsphenamine in weekly doses for a period of three weeks, which would just fall short of producing jaundice. After the three injections, the meat-fat diet was continued for a fourth week. Van den Bergh tests were made every other day during these four weeks. They were consistently negative on all dogs. Then the diet was switched to high carbohydrate. It was hoped that this sudden "flooding" of the animals with carbohydrates would add an unsupportable burden to

livers already subjected to three large doses of arsphenamine. Van den Bergh tests were again made every second day for a period of a week. None of the animals became jaundiced. The dogs were then sacrificed together with a comparable series of control animals which had received identical quantities of arsphenamine, but had remained exclusively on a meat-fat diet. Autopsy and microscopic study showed no necrosis in any of the animals. Eight animals were used in this experiment, four in each series.

Positive results in an experiment similar to that above still remain a possibility, and the work should be repeated. To obtain positive results the dogs should be loaded with the maximum quantity of arsphenamine that just falls short of producing jaundice. Such a desideratum could be obtained only by trial and error.

Voegtlin (5) in 1925 showed that the toxic effects of "arsenoxides" were considerably diminished in rats by the previous injection of glutathione. He has recently repeated and confirmed this work with a pure crystalline reduced glutathione (6). He hypothesized that the *SH* radical was the arsenic receptor and in such a combination the arsenic was rendered relatively non-toxic. The amino-acid, cystine, also contains the *SH* radical. By feeding cystine to his rats, Voegtlin was able to reduce the toxicity of trivalent arsenicals, although not to the same degree as with glutathione.

The results we have obtained with diets is consistent with the above facts, the meat-fat diets presumably containing more cystine or cystine derivatives than the carbohydrate diet. Cystine added to this carbohydrate diet should, therefore, provide the otherwise absent sulphydril radicals, and render the diet as efficacious in preventing liver necrosis as are meat and fat diets.

We are able to report experiments on twenty dogs. Eight of these had cystine added to their diets (by stomach tube) before the administration of arsphenamine. Two animals received arsphenamine in which had been dissolved (by the addition of excess NaOH) three grams of cystine. The remaining ten dogs were kept as controls and received no cystine. All twenty dogs were given the high carbohydrate diet.

The dogs were given the high carbohydrate diet for four days preceding the injection of arsphenamine. Three hours before the injec-

tion eight of the animals were given 3.0 grams of cystine each by stomach tube. Then, a single large dose of arsphenamine (0.03 gram per kilo), was injected into all twenty animals. In the case of two dogs, 3 grams of cystine were dissolved in their portion of arsphenamine solution, as mentioned above.

Autopsy and microscopic study showed marked liver necrosis in all twenty animals, with extreme necrosis in several. There were no differences observed between the two series of animals, those having received cystine showing just as marked necrosis as those which received no cystine. The two dogs which received their cystine intravenously also exhibited marked liver injury.

Glutathione may be more efficacious, as it certainly was in Voegtlin's experiments, and should be tried.

CONCLUSIONS

1. High fat and high protein diets provide the maximum protection against the liver injury caused by arsphenamine. Of these two diets, the former is more efficacious.

2. High carbohydrate diets afford maximum susceptibility to the liver injury caused by arsphenamine.

3. Starvation is important as a predisposing factor toward the liver injury caused by arsphenamine.

4. Cystine added to the diet or given intravenously does not increase the protective action of carbohydrate diets.

I wish to express my appreciation to Dr. Arnold R. Rich for many helpful suggestions and criticisms.

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ACID-FAST BACTERIA[1]

Their Relation to Disease and the Need for More Knowledge

WM. CHARLES WHITE, M.D.

Washington, D. C.

In the progress of bacteriology, studies seeking to explain the varying pathogenic properties of closely related strains of bacteria within a bacterial family have in a measure superseded the enthusiasm for isolating new species. Newer methods of chemistry, physics and biology have been enlisted to obtain from each strain pure chemical units which may be compared with each other as to biological significance and so possibly teach us the underlying principles of pathogenicity and saprophytism. The trend of modern thought is evident when one reviews the recent work on the pneumococcus, streptococcus and diphtheria families. It has perhaps been most systematically exemplified in the last seven years' work on the mycobacteria family carried on by the Committee on Medical Research of the National Tuberculosis Association (1). This committee's study of the biological action of specific chemical units derived from strains of mycobacteria has brought to light a number of features which serve to clarify our knowledge of the mycobacteria family.

Type strains of this family, grown on a common synthetic medium, in common glassware, and when only common chemicals of known characteristics were used, have yielded chemical units by common methods of chemical analysis which give definite biological responses when tested on normal and tuberculous animals. These chemical units are of two types; those common to all members of the family, and those specific for the living chemistry of the different strains of the family.

The mycobacteria family in most classifications is listed as contain-

[1] Delivered as the De La Mar Lecture of November 25, 1930 at the School of Hygiene and Public Health, The Johns Hopkins University.

ing some nineteen species, eleven of which are listed as pathogenic and eight as saprophytes in the following table:

Key to the species of Mycobacterium (2)

A. Parasitic in warm-blooded animal	
1. Parasitic in man	1. Mycobacterium tuberculosis (hominis)
	2. Mycobacterium leprae
2. Parasitic in bovines	3. Mycobacterium tuberculosis (bovis)
	4. Mycobacterium paratuberculosis
3. Parasitic in birds	5. Mycobacterium avium
B. Parasitic in cold-blooded animals	
1. Parasitic in fish	6. Mycobacterium piscium
	7. Mycobacterium marinum
2. Parasitic in frogs	8. Mycobacterium ranae
3. Parasitic in snakes	9. Mycobacterium tropidonatum
	10. Mycobacterium thamnopheos
4. Parasitic in turtles	11. Mycobacterium chelonei
C. Saprophytes	
1. Found on skin and genitalia of man and animals	12. Mycobacterium smegmatis
2. Found in butter	
a. Litmus milk alkaline	13. Mycobacterium butyricum
aa. Litmus milk shows reddish-yellow ring	14. Mycobacterium berolinense
aaa. Litmus milk becoming dirty yellowish-brown	15. Mycobacterium freiburgense
3. Found in manure	16. Mycobacterium stercoris
4. Found in hay	17. Mycobacterium phlei
5. Found on grain	18. Mycobacterium graminis
6. Found in soil	19. Mycobacterium alluvialum

But this is only a small part of the story for it is known by all who work with these organisms that the strains within the species are almost as variant as the animal source from which the individual strain is cultivated. The present methods of classifying them by acid production in a given culture medium or by pathogenicity for rabbit and guinea-pig are very uncertain. Van Es (3) has shown that some strains of the avian bacillus are quite as pathogenic for the rabbit as are some of the bovine strains, so that one could not always be sure that because a strain was pathogenic for rabbits it should be classed as a bovine type (table I). There are also in all typing studies that have been made by past methods from 2 to 5 per cent of the strains cultivated which are classed as mixed strains or atypical strains (4).

RELATIONSHIPS OF THE MYCOBACTERIA FAMILY

On the one hand the mycobacteria are related to the actinomyces by their occasional branching and staining properties. There are known over seventy of these actinomycetes and more than two-thirds of these live in the soil. I shall refer to this relationship again in another paragraph.

The mycobacteria are also related to the corynebacteria by their production of metachromatic granules. Interest in these granules has lately been greatly intensified by the studies of M. C. Kahn (5).

TABLE I

HOST SPECIES	BACILLARY TYPES			
	Bovine	Human	Avian	Atypic
Man	++	+++	+*	*
Bovine	+++	0*	+*	(?)
Swine	+++	+*	++	+*
Horse	++	(?)	+*	+*
Sheep	++*	0*	(?)	(?)
Goat	+++	0*	0*	(?)
Cat	++	0*	+*	(?)
Dog	++	++	0*	(?)
Cavia	+++	+++	0	*
Rabbit	+++	+	+++	*
Fowl	0	0	+++	*
Parrot	+++	+++	++	*

+++, full virulence; ++, moderate or conditional virulence; +, light virulence; 0, avirulence; *, data insufficient for definite conclusions; (?), unknown.

He has shown that they are an important viable part in the life history of a single bacillus.

GROSS DISEASE PICTURES OF PATHOGENIC STRAINS

A picture of two disease conditions produced by this family of bacteria, namely, wasting, and hypertrophy of skin cells, are typified by Jöhne's disease and by leprosy (Figs. 1 and 2). You are all familiar with the picture of tuberculosis of the lungs showing in its advanced stages great wasting, and of lupus showing also hypertrophic skin changes. The wasting and ex-succus condition in chickens caused by

certain strains of the avian type of bacillus has been particularly noted by Sabin (6), who suggests that the disease tuberculosis may be divided into a number of clinical pictures dependent on different

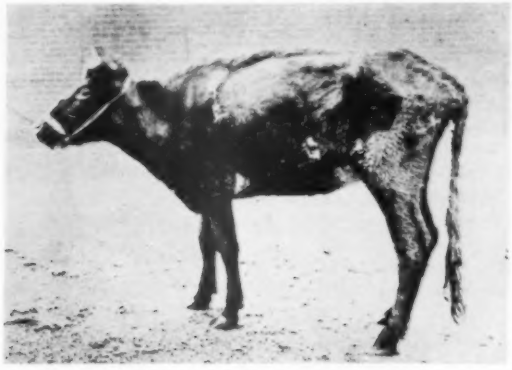

FIG. 1. WASTING IN JÖHNE'S DISEASE

FIG. 2. SKIN CHANGES IN LEPROSY

strains or upon the different chemical units dominant in a given bacterial strain.

Another characteristic of the diseases caused by pathogenic mycobacteria is the temperature response which the body makes not only

to infection itself but after infection to the parenteral introduction of certain substances isolated from the bacilli (7). This is exemplified by what we call the general tuberculin reaction and by the characteristic temperature of an advanced case of tuberculosis in man. We now know two clean-cut chemical units taking part in this temperature reaction, a protein and a polysaccharide.

LIFE HISTORY OF MYCOBACTERIA FAMILY

For study purposes the life history of the mycobacteria may be divided into three phases: (*a*) Live history in nature; (*b*) Life history in animals; (*c*) life history in the test-tube.

Life history in nature. No family of bacteria has a wider spread in nature than the mycobacteria. The saprophytes occur as the timothy grass branch, the dung branch and the grain and soil branches, and also on the skin and genital organs of man and animals, as the smegma bacillus. One can scarcely doubt that there is a certain relation between the fact that these saprophytes are found on the skin and genitalia of man and animals and on plants and in soil. Yet no one, so far as I know, has concerned himself with the correlation of these observed facts. I would suggest as a working theory that all animals ingest acid-fast bacilli with their plant food and that there is a surviving residual strain at the fecal exit which, after the passage through the intestinal tract and all the varying factors met there, finds a food supply and physical conditions suitable to existence. Probably a spread occurs from the anal opening only so far as the food supply and physical conditions are adequate. Certain strains may overgrow in this locality of spread dependent entirely on physical conditions such as temperature, moisture, light rays and especially food supply. They may pass from the genital and anal locations in a continuous supply in the urine, feces and sweat to the soil and so, after a cycle there, back to the bodies of other animals.[2]

[2] It is notable that in an endeavor recently to cultivate a strain of smegma bacillus in one of our Veterans' Hospitals none could be found. This was ascribed to the cleanly habits generated in the modern bath-room. This attempt was made by Dr. Corper because there was no strain of smegma bacillus the history of which was accurately known. In laboratory work the carelessness of accepting strains without assurance of their history, purity, and pathogenicity has had a sad example in the recent calamity at Lübeck with BCG.

In the soil. Few students have followed the life history of these strains or of the other strains of mycobacteria in that great respository of all germ life—the soil. From the time of Koch one of the chief efforts of students of bacterial life has been to isolate and follow the life history of pathogenic and other organisms in pure culture in the test-tube, and by injection to follow further their life history in different animals. In doing this they have neglected the other great one-third of the cycle in nature where they live in competition and communism with all other living things. There are, however, brilliant examples of the importance of study of bacteria in relation to conditions nearer the soil than man, in malaria, rocky mountain spotted fever, tularemia and plague. In the soil, for example, bacterial life may meet such variations of temperature, moisture, food supply, sunlight, communal life, or intermediary host as to determine the survival and overgrowth of virulent or avirulent, pathogenic or saprophytic strains, and this may be the realm of explanation of many of our obscure disease cycles.

Take for example the recent work of Armstrong (8) who demonstrated a great increase in virulence caused by selecting a heat-resistant strain of vaccine virus. It is possible that our outbreaks of smallpox this fall may be associated with the heat and drought of the past summer. Or take the suggested very low temperatures found necessary by Spencer (9) for the virus of rocky mountain spotted fever in its life history in the tick to establish its virulence for man.

Returning now to the mycobacteria, Schalk (10) recently found that the avian tubercle bacillus cast upon the ground by infected chickens survives two Minnesota winters. Infection of uninfected chickens and pigs fed on the plant life of this ground occurs in the two following summers after closing the plots during the intervening winter. The knowledge also that organisms dried at low temperatures can be kept virulent for long periods is significant in this relation. Also, the carrying of infections of mycobacteria by animals not themselves infected has been clearly shown by Schalk (11). Rats and mice taking in their food the droppings of tuberculous chickens will carry tubercle bacilli to other clean chambers and infect clean pigs and chickens whose food in turn has become infected with the droppings of these rodents. These factors can scarcely fail to have importance

in the epidemiology of our pathogenic mycobacteria and may possibly explain our inability absolutely to control infection by them even under the most rigid methods which we employ with cattle.

The subject of the importance of the life cycle of our acid-fast family in the soil has recently been emphasized by Walker (12) in connection with leprosy. Walker thinks, and with reason, that certain strains of Hansen's bacillus during their life in the soil, exposed to physical or secondary host changes, pass through some modification, as for example, by survival or overgrowth of peculiar strains, which bring to the front those capable of life in the monocytes of the skin of man or rat. The same possibility applies to those peculiar strains of the tubercle bacillus, responsible for lupus. It is known that we can not produce a disease in man or animal comparable to lupus, and that all strains cultivated from lupus lesions have peculiarities of their own (13).

Referring now for a moment to that curious group of strains known as the "paratubercle bacilli" which inhabit in disease the monocytes of the ileum and caecum of cattle and which are responsible for Jöhne's disease. These strains, Twort (14) found, must have in the test-tube as a requisite for growth some dead acid-fast bacilli. We know comparatively little about their life history in nature. It is true that they are poured in great numbers upon the soil in the feces of cattle and that in many countries they cause great loss of animal life. One fact is clear: they are not able to start *de novo* their synthetic life history *in vitro*. Some relative of their own must do a certain service for them. What this service is should be a problem of easy solution for the modern chemist.

Other strains must be mentioned only to point out our lack of knowledge: for instance, those somewhat rare strains found in snakes, turtles, fish and other cold-blooded animal groups with which Aronson (15), of the Phipps Institute, and Rabinowitsch (16) in Berlin, have continuously worked. These certainly occur in nature but what underlies their strain specificity we have little notion. It is possible that the comparative chemical studies now under way may lead us to the key of their peculiarities.

We have recently received from Dr. Hastings of Wisconsin a strain of that interesting species cultivated from the tissues of cattle and

possibly underlying that very vexing problem of no-lesion reactors which are found in the work for the prevention of tuberculosis in cattle. We shall submit this strain to the comparative chemical and biological study to which we are submitting the type strains of the human, avian and bovine tubercle bacillus, the timothy grass bacillus and certain strains of the so-called lepra bacillus. Dr. Hastings' strain presumably is transferred directly from the soil to the skin of cattle and grows there.

Function. Wide distribution of this family of bacteria in widely varying strains is then the outstanding feature of this sketch of our present knowledge of their life in nature. So far as I know, no effort has been made to ascribe to them a useful function and yet one can scarcely avoid the belief that from their ubiquity they have one. It is the hope that the comparative chemical study may open this door to our knowledge.

Life history in animals. Laying aside the smegma bacillus which grows upon the surface and the mycobacterial strains in the intestinal tract, our main interest moves at once to the many pathogenic strains of the mycobacteria family. The chief feature of the life of these strains within the body of animals is their relation to the monocyte of the mesoblast group of body cells.

Host-parasite relationship. It seems to be true that in order to produce disease a mycobacterium, after gaining entrance to the body, must gain entrance also to a monocyte (17) in what we call a susceptible animal. A tubercle is made in its early stages of groups of monocytes harbouring tubercle bacilli. In the rat the process never goes beyond this stage. In other animals all grades of progression are seen up to the stages of caseation, softening and discharge. Caseation and softening are often coupled with calcium deposit in some animals. The bacterial strains have, however, within their composition in their phosphatid fraction a substance isolated by Anderson (18) and called by him "phthioic acid" which Sabin (19) has shown possesses the property of stimulating the multiplication of the host cell or monocyte. That is, a cycle of relationship has been shown to exist between a parasite and its host cell whereby the production of new host cells is stimulated for the parasitism of the bacillus by an inherent property of the parasite. Whether this is active only on the death of the parasite generations is not yet known.

There is, I believe, as true a host-cell parasite relationship existing between the monocyte branch of the mesoblast family of cells and the strains of mycobacteria as exists between the red blood cells and the strains of the malarial parasite family. This, I believe, at the present stage of our knowledge may be called the monocyte-mycobacterium commensalism. Probably until we understand the living chemistry of each contracting party in this commensalism we shall not have found the secret of cure of these diseases.

That this commensalism is present in widely varying animal families to a single strain of mycobacterium has recently been shown by a beautiful piece of work. Long and Vorwald (20) have followed the changes caused in a series of animals from the mouse to the monkey, by the introduction of the same dose per kilogram of body-weight of the same strain of bacillus, the human strain known as H37. They have shown that there is always a monocyte-parasite relationship as a primary factor. This feature of the constant relation of specific host cell and parasite in all animals, and the existence in the susceptible animal of an inherent factor in the host cell which enhances the communal sympathy of development of the strain of parasite, is of fundamental importance. It is this elusive chemical factor of the cell host, in the animal strain, and of its location in the individual animal, which, I believe, once discovered, will bring the conquest of this problem within our grasp. One possible approach to it is through the specific stable chemical units that can be isolated from the different controllable strains of this acid-fast family.

It is not to be thought that this is the only commensalism which the monocyte entertains. We known that a similar one exists with the brucella family. It would be very unlikely that common chemical units should not exist widely in bacterial families. The brucella family has some features in common with the mycobacteria, for example, the relation between abortion in cattle and the avian strains of the tubercle bacillus as shown by Plum (21). There are certain protein, carbohydrate and phosphatid chemical units more or less common to all animal life and therefore it is likely that there are some common factors in all bacterial life. But it is the rare chemical units responsible for the specificity of the peculiar monocyte-mycobacterium commensalism that we must seek for and isolate and study in our search for strain specificity.

Properties of the monocyte. In seeking the reason for the specific relationships of these two living cells we must first examine the host cell or monocyte. There are grave obstacles in the way of studying the chemistry of the monocyte because of the difficulty of obtaining it in pure culture. It is, however, a highly differentiated mesoblast cell possessing characteristics common to all amoebae wherever found. It does not differ in these respects simply because it carries out its life in the fluids of an animal body confined by a skin boundary. Its tendency to multinucleation, its relation to bacterial life, its power of free motion, may be cited as examples of what I refer to. That it has a specific internal chemistry of its own differentiating it from other cells of the body is shown by pictures which it gives while it is still alive, with one azo dye (janus green) and one quinone-amide dye (neutral red) (22).

Origin. The origin of monocytes in the body has lately been presented in a new light by C. S. Forkner (23). He ascribes their origin chiefly to the lymph nodes but also finds them arising in the spleen, Peyer's patches, subcutaneous connective tissues, lungs and omenta in the rabbit. They have not been fully worked out for other animals.

Round of activity. Their chief round of activity in the body is from their places of origin probably generally towards the liver. They probably operate in the liver as the Kupffer cells. I know this is a much debated point and I offer it only as my own conception. In the lung they are certainly very abundant and perform some function there. It will be seen that their stations of origin or manufacture and their chief functioning stations correspond with the sites of disease processes of the acid-fast group in the bodies of different animals. It is only fair to suppose that origin, round of activity, and function differ in different animal strains and in different individuals of the same strain of animal under varying conditions.

Certain correlations or contrasts must then be established, on the one hand, between the chemical processes of these monocytes in their function in various animal strains, and in the different organs of the same animal of the same strain and, on the other hand, with the chemistry of the peculiar strain of acid-fast organism that grows in a given location after gaining entrance to the individual animal.

Function. Of their function in this round of activity we are not

very sure. Certain observations would indicate, however, that they are in some way concerned with fat metabolism and burning, one of the most significant of the observations being that in the rat where monocytes are acting as host cells to the tubercle bacillus the lungs become literally choked with these cells swollen with fine fat globules and bacilli. This fact and the power which these acid-fast organisms have of manufacturing up to one-half their body-weight with highly organized fats and waxes would seem, in theory at least, to make them very closely associated in their living chemistry with fat metabolism (24).

Selectivity of strains. That selectivity of strains for tissues occurs you have already seen exemplified in lupus, leprosy, and Jöhne's disease. There is, however, a phase of this selectivity coupled in some way with dosage. I have a strain of bovine bacillus given to me by Vallée of Alfort. It is one of the few bovine strains that will grow on our standard medium. If this strain is given to rabbits by the ear vein in doses of 1/200 of a mgm. per kilo it produces the ordinary miliary general type of disease, but if this dose is reduced to 1/2000 of a mgm. per kilo the rabbit does not show sickness for many months and then there are found gross caseous lesions of the testicles with comparatively little tuberculosis elsewhere. These testicles may be 4 inches by 2 inches in size. This is part of some work done by M. I. Smith (25) at the National Institute of Health. Of what this dose factor consists I have no explanation, but I am sure that when our task is done we shall know the characteristics of strains which lead them to grow in the monocytes of a selective habitat in the body. Methods of study of these diseases by introducing stock strains into animals in varying doses by injury is of course crude and often results in such gross disease that we are yet only at the beginning of the problem of the natural course of events in the natural epidemiology of this family of bacteria.

Life history in the test-tube. Obviously the possibility of cultivating pure strains on a common medium has opened a new avenue to the study of tuberculosis and other disease conditions. The search for the pure chemical units in the strain responsible for its peculiar biological properties is moreover in line with all that is best in modern biological chemistry. I will confine myself chiefly to the results of such efforts and say little or nothing of the cultural characteristics on different media in the test tube with which you are all more or less familiar.

Methods of chemical study. Since many of the strains of this one family will grow on a common simple synthetic medium, Long's medium,[3] large quantities of bacteria of any chosen strain can be made available for comparative chemical study. A large quantity of bacteria as a primary unit is necessary for this chemical analysis because the terminal active chemical substances are often present in such small amounts that after finding their presence, unless enough is available for further chemical and biological study, much of the labor has been wasted. I need only refer to the small production of insulin, by the minute islands of Langerhans, as a powerful agent in the body to indicate how minimal is the amount required of some of the most active chemical substances in living conditions. It is essential also to establish a base line of one strain for such a comparative chemical study and to remove all the variants possible at the beginning of the study. For example, the chemical ingredients of the medium and the glass must be constant.

When then a medium of known chemical composition is used, every new organic substance appearing in the flask of growth of a given bacillary strain must have been made by the development of that strain and so exact quantitative data can be drawn. The same laws hold good so far as possible for the comparison of the biological data obtained by the injection of these isolated substances into animals. The biological tests are the final guide to the chemist as to his future procedure and a base line must be fixed and as many variants as possible removed.

Common factors. With this introduction and partial repetition I shall describe a few of the common factors and contrasts already known for the family of mycobacteria, particularly the tubercle bacilli.

[3] Long's medium:

Asparagine	5.00
Ammonium citrate	5.00
Potassium acid phosphate	3.00
Sodium carbonate (anhydrous)	3.00
Sodium chloride	2.00
Magnesium sulphate	1.00
Ferric ammonium citrate	0.05
Glycerol	50.00
Water, distilled Q. S.	1,000.00

From the time of Koch's picturesque and confident announcement of a cure for tuberculosis before the Berlin Congress for Medicine interest has never died in his supposed curative agent, tuberculin. This is a glycerine extract of the tubercle bacillus and is still used widely as a diagnostic agent for tuberculosis. Rising and falling waves of enthusiasm have attended its use in the hands of clinicians. It has not yet reached the status of a settled question. In 1907 von Pirquet presented to the world the tuberculin reaction and the whole question of allergy which later became involved with the problem of immunity. Within the last few years allergy and immunity have been clearly separated by Rich (26) Branch (27) and others. The existence of allergy in an animal is mainly evidenced by an inflammatory reaction of the skin or other tissue to the local injection of a substance, usually of a protein nature, derived from the organism which is supposed to be living as a parasite in the body examined. Allergy varies, as one knows, with the animal. It is marked, for example, in man, cattle and guinea-pigs for tuberculin. It is almost impossible to elicit it in the rabbit and rat for the same agent.

The substance in the tuberculin responsible for this skin reaction in the sensitive animal has been greatly purified by the work of Seibert (28). In fact she probably has produced it in crystalline form. A very pure commercial product under the designation MA-100 from the several type strains of acid-fast bacilli is now made by the Mulford Laboratories of Sharp & Dohme at Glenolden (29). A curious thing is that when this substance is pure it is apparently one of the common protein fractions for the acid-fast family. At least the difference in the strains is in the main a quantitative rather than a qualitative one. There are, however, evidences of a qualitative difference in a small group of the patients on whom biological tests have so far been made. It is significant in the recent work of Funk and Huntoon (30), in testing the skin reaction of patients with these pure proteins, that upwards of 95 per cent of the patients give a skin reaction to the protein fraction of the timothy grass bacillus, a non-pathogenic strain of the family, if the quantity used be proportionately increased so that a skin reaction unit is made the basis of the dilution. This in a measure may reduce our confidence in the specificity of this widely used clinical testing agent. The explanation of this quanti-

tative difference is difficult and will be reported on when more facts are known.

It is a sad commentary on human endeavor that in much otherwise excellent laboratory work almost any tuberculin is considered good enough. And yet one can scarcely conceive of a more complex material. We know that market tuberculins differ at least 400 per cent in strength. We have no satisfactory way of standardizing it and in fact little attempt is made to standardize each batch offered for sale or use. When attempts are made, it is standardized on its guinea-pig-killing properties and then used for testing allergic skin reactions. It is known from the work of Dorset (31) and the writer (32) that there is probably no definite relation between these two biological reactions if we could have clean chemical units. One of the guinea-pig-killing fractions of market tuberculin is probably a very stable polysaccharide substance unchanged by boiling, which gives no skin reaction. As stated above, the skin reaction is due probably to a water-soluble protein fraction that can be prepared in pure form. The two guinea-pig-killing fractions contained in old tuberculin kill in different time periods and depend in part on the extent of the disease in the animal used.

In other words the tuberculin reaction, so-called, is a group of reactions, each due to a specific substance in the tuberculin and we have no more justification for construing the phrases, local, focal, and general reactions as if they were different phases due to a common substance in tuberculin than we have for continuing to speak of allergy as part of the immune reaction.

It should be possible very shortly to furnish in pure form of standard quantitative strength each fraction of the different bacillary strains that has a specific biological reaction associated with it, so that clinicians may read with some accuracy their clinical results.

It is not surprising that there are chemical units common to all strains of this family, such as this water-soluble protein and many others of which we know in the fat and wax and carbohydrate chemical groups. The protein responsible for the reaction is widely distributed in each bacterial cell apparently in varying combinations with other chemical units and can be prepared from various residues after extraction with different solvents. The same is true for some common carbohydrates and fats.

Varying factors. The widest differences are probably in some of the carbohydrates fractions. This became apparent first in the medium of growth when followed weekly in test bottles (33). The quantities of reducing sugars obtained before and after hydrolysis in the different type strains are very marked.

Shortly after this work Anderson (34) prepared a polysaccharide from the phosphatid fraction of the human tubercle bacillus which I found (35) to have marked guinea-pig-killing qualities. The death of the tuberculous guinea-pig occurred often within four hours after the injection of a dose of 10 mgm. per kilo of the polysaccharide and was preceded by a rapid fall in temperature and a change in the lipoid

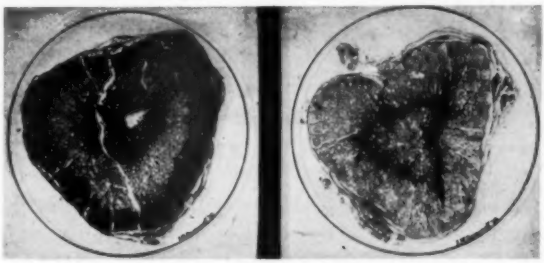

FIG. 3. *a*, NORMAL LIPID CONTENT STAINED WITH OSMIC ACID; *b*, CHANGES IN LIPID CONTENT IN GUINEA PIG KILLED WITH POLYSACCHARIDE

substance in the adrenal gland of the pig, shifting the lipid content of the cells of the adrenal from the normal cortical and central zones to a middle zone. Also, there were changes in the cells of the central zone of the gland and great swelling of the central vein (Fig. 3).

I can not go into theoretical explanation of this at this time. The next step was the correlation of this work with that of Laidlaw and Dudley (36) in England, and with Müller (37) in Dr. Zinsser's laboratory, who found a similar substance that could be precipitated by a serum prepared by injecting dead defatted bacilli into rabbits. Three years previously Reichel had prepared a serum by injecting defatted bacilli into a horse while pursuing another line of work. This serum he had kept and it was now found to precipitate this polysaccharide in very dilute solutions. In fact dilutions as high as 1 in 2 and 3 mil-

lion parts of the sugar would give a precipitate with this serum. A similar polysaccharide was later isolated by Masucci (38) and by Johnson and Renfrew (39) from the medium of growth.

As the work progressed, it was found that the polysaccharide produced by the same method from the timothy grass bacillus was also precipitated by this serum but that those from the avian and bovine strains of the tubercle bacillus were not precipitated, nor has Reichel been able yet to produce a serum by the injection of a homologous strain into a horse which will precipitate the sugars from the latter strains. The serum of the human strain injections, however, precipitates a polysaccharide prepared from the bovine strain by Heidelberger, so that there is again a common factor as well as a specific factor in the strains used.

These polysaccharides are non-antigenic, produce no skin reaction, are soluble in all proportions in water, are very stable, requiring boiling by strong acids to hydrolyse them. They are excreted almost quantitatively in the normal rabbit urine and yet are severely toxic for the tuberculous guinea-pig.

Anderson (40), Heidelberger (41), and Renfrew (42), pursuing their analysis of these sugars further, find them phosphorus groupings of inosite, mannose, invert sugar, and d-arabinose. This is only the third time d-arabinose has been reported found in nature. It would seem as if in this very sensitive field we should light upon one link of our specific difference in these strains. There are other sugar groupings in other fractions of the cell, for example, in the nucleic acid fractions. But this work has not proceeded far enough for report at this time.

We feel sure that this stable poisonous fraction is circulating in the serum of the sick animal, but failure has so far followed our efforts to exhibit it. If there, it is probably bound or adsorbed. Efforts are still being made to find it, for the idea has possessed us that this type of substance is in some way responsible for some of the symptoms of the sick tuberculous, especially the peculiar temperature curve.

It has seemed possible that if we could show its presence in the blood and aid its hydrolysis to non-poisonous substances we might in part alleviate the suffering of the sick. Last year by chance I found an organism that would readily hydrolyse this complex stable polysac-

charide, and this Heidelberger is now studying with the above object in mind.

I shall refer only briefly to one other wide difference in the chemical units of the many that exist. Table II is one of Anderson's (43) recent tables of comparison of three of the type strains used in the chemical work. It will be apparent that in gross amounts there are wide differences in the phosphatide fraction, in the acetone-soluble fat, and in the total lipoids of the three strains. Anderson has found that the chemical units in the phosphatide fractions of the three strains differ in several particulars and Sabin and her co-workers (44), es-

TABLE II

Lipoids and other fractions separated from bovine, avian, and human tubercle bacilli

	TYPE OF ORGANISM					
	Bovine, strain 523		Avian, strain 531		Human, strain H-37	
Approximate number of cultures	1700		2000		2000	
	grams	*per cent dry bacteria*	*grams*	*per cent dry bacteria*	*grams*	*per cent dry bacteria*
Phosphatide, crude	60.5	1.53	79.7	2.26	253.1	6.54
Acetone-soluble fat	131.7	3.34	77.3	2.19	240.0	6.20
Chloroform-soluble wax	336.0	8.52	379.5	10.79	427.0	11.03
Total lipoids	528.2	13.40	536.5	15.26	920.1	23.78
Polysaccharide	43.0*	1.09	36.1	1.02	33.9	0.87
Dried bacterial residue	3,370.0	85.50	2,942.7	83.71	2,902.0	75.01
Total dry bacillary material	3,941.2		3,515.3		3,868.5	

* This figure is included provisionally.

pecially Doan (45), have discovered biological differences. So far as this work has been finished, the biological response in monocyte numbers is different in the reaction of rabbit and guinea-pig to the injection of the phosphatide from different strains and also in the internal chemistry of the individual monocytes as shown by their morphology after such injection.

The phosphatide fraction is antigenic and when tested against the sera of animals injected with phosphatide of the different strains (46) there is a marked difference in the dilution at which precipitation occurs with the homologous and heterologous phosphatide. This

suggests again that there is a fraction common to all strains but also a specific fraction for each. Both must be separated and studied individually.

I can not take the time to describe the other fractions but I have said enough to indicate the course and possibilities of such a comparative chemical and biological study as was mentioned at the opening of this paper.

NEED FOR MORE KNOWLEDGE

I am sure it impresses you, as it does me, that here is a field in which we need more exact knowledge. The diseases produced by the acid-fast bacteria represent the greatest economic problem of the human race, not only for man, but for one of his major industries, that is, his animal food supply. The pitiful appeal in the eyes of those dying by wasting from tuberculosis (and we still have over 150,000 a year in the United States) presents the saddest expression of our failure. No one can be proud of a cure that takes on an average two years out of the most active period of our youthful victims.

I trust that this description of a new effort to obtain knowledge with the assistance of skilled workers in many scientific fields will draw not only your sympathy but your honest constructive criticism, that we may straighten our paths of study in such a way as to lead us with the least possible loss of time to our goal—the control and cure of the group of diseases caused by the acid-fast family of bacteria.

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ON THE BOUNDARIES OF THE SINO-AURICULAR NODE AND THE ATRIO-VENTRICULAR NODE IN THE HUMAN HEART[1]

HELEN B. TAUSSIG

From the Cardiographic Laboratory of the Johns Hopkins Hospital and University

This paper is concerned with the relation of the S-A node and the A-V node to the auricle. The literature on the specialized tissue of the heart is so vast that a complete review of the subject is far beyond the scope of this report.

Most of this work has to do with the specialized tissue itself. It is concerned with the anatomical position of the two nodes; with the histological structure of the nodes, and their physiological function. Comparatively little has been written on the boundaries of these nodes, *i.e.*, on the transition from the S-A node to the auricle and from the auricle to the A-V node.

In this respect the study of the specialized tissue of the auricles stands in striking contrast to that of the ventricles. The fanning out of the right and left branch of the Bundle of His to form a network in the interior of the ventricles is, of course, well known.[2] Years ago Tawara (46), in his study of the specialized tissue, demonstrated that the transition from the Purkinje cells to the cardiac muscle cells was so gradual that it was impossible to tell where the one began and the other ended.

Our knowledge of the boundaries of specialized tissue of the auricle is much more limited. As a preliminary it is important to understand clearly what is meant by the "specialized tissue." The name "Purkinje cell" was first applied to the large cells found in the lower part of the Bundle of His. The term is now frequently used to apply to all the specialized tissue in the heart. It is well known that the cells of the specialized tissue differ appreciably in different parts of the heart

[1] Submitted for publication January 17, 1931.

[2] This is shown in all the textbooks of Anatomy and beautifully demonstrated by DeWitt (10) and Cohn (7).

and also in different species. Many studies have been made and many different points have been emphasized in regard to cell structure, glycogen content (1, 3, 32), sarcoplasm, mitochondria (31, 45), etc. Hoffmann (12), Kölliker (26), Marceau (30), Obermeier (34), Tandler (44), Tang (45), Thorel (47, 48, 49). Todd (50), Van der Stricht (51), describe the cells of specialized tissue as follows:

(1) The cells have relatively large, oval or round, central nuclei. Frequently there are multiple nuclei.

(2) The nuclei are surrounded by a relatively large zone of clear cytoplasm.

(3) The outer parts of the cells are composed of long fibrillae which interweave and anastomose[3] freely from cell to cell. In these cells the longitudinal striations are much more pronounced than the cross striations, though the latter are distinctly visible.

Because of the variation in these characteristics some authors, Kölliker (26), Obermeier (34), Todd (50), Van der Stricht (51), Tandler (44), differentiate three kinds of Purkinje cells.

(1) The large square cells with twin nuclei and a clear zone of cytoplasm surrounded by a narrow curiously striated zone.

(2) The long slender cells with oval nuclei surrounded by a clear zone of cytoplasm and at the margin of these cells long branching fibrillae whose longitudinal striations are more marked than the cross striations.

(3) The curious "nodes" or "knots" of interweaving fibrillae which have no obvious nuclei, but are merely a mass of tissue forming a stellate pattern.

Todd and Van der Stricht (51) differentiate the embryonic type of Purkinje cell, the adult type of cell, and the transitional cells.

(a) The cells of the embryonic type are polyhedral or with the long axis somewhat exceeding the diameter. The cells contain several nuclei lying close together surrounded by a granular zone of cytoplasm and a cortical fibrillar area. The myofibrils show transverse striation, but the longitudinal striation is always much more pronounced than the transverse.

(b) The adult type of specialized cell is much longer and often more bulky than the embryonic cell. The nuclei tend to be distributed laterally and through the length of the cell. The cells divide in such a way that a single axis becomes bipartite or tripartite. This effect is produced mainly by the myofibrillae of the cortical

[3] Hoffmann (12) considers this interweaving and anastomosis of the fibrillae one of the most characteristic features of the specialized tissue cells.

layer invading the central granular sarcoplasm and thus accentuating the multipartite character of the Purkinje cells.

(c) The transitional cells represent the gradual transition from the true Purkinje cell to the cells of true cardiac muscle.

With these points clearly in mind, let us return to the main subject of the paper, the boundaries of the S-A node and the A-V node. Let us take the A-V node first because it presents a simpler problem.

The A-V node is readily demonstrable in the calf and sheep heart. It lies in the right interauricular wall below and slightly beyond the foramen ovale and close to the mouth of the coronary sinus. If one cuts the right auricle so as to expose the interauricular wall and gently pulls on the coronary sinus, one can discern the node. By removing the endocardium and following up the main stem of the Bundle of His, it is easy to dissect it out. It is clearly seen because it is whiter and more glistening than the surrounding muscle tissue. If one does this, one is impressed by the fact that, although the main bulk of the A-V node is sharply separated from the auricular muscle, there are very definite branches which extend from the node into the auricle. S. Kent (17, 18, 19) has written several reports on the right lateral branch of the A-V node. Curran (9) in 1909 emphasized the stellate form of the node and showed that these branches had definite positions in the calf heart. Although it is easy to demonstrate these branches, it is difficult to follow them for any great distance. They soon taper off and become lost in the surrounding connective tissue and cardiac muscle.

The striking and interesting point in the human heart is that, if one cuts serial sections parallel to the A-V node, one sees precisely the same phenomena. The main tissue of the A-V node is sharply separated from the auricular muscle. Indeed in most places there is a wall of connective tissue between the two. Nevertheless, there are places along the border of the A-V node where this connective tissue disappears. In such places one can follow strands of specialized tissue as they extend out into the auricle. At first the tissue is composed of typical nodal cells, (this is shown in figure IA). Gradually the nuclei become elongated, the zone of clear cytoplasm about them decreases, the interweaving of the fibrillae becomes less prominent, the longitudinal striations less pronounced and the cross striations

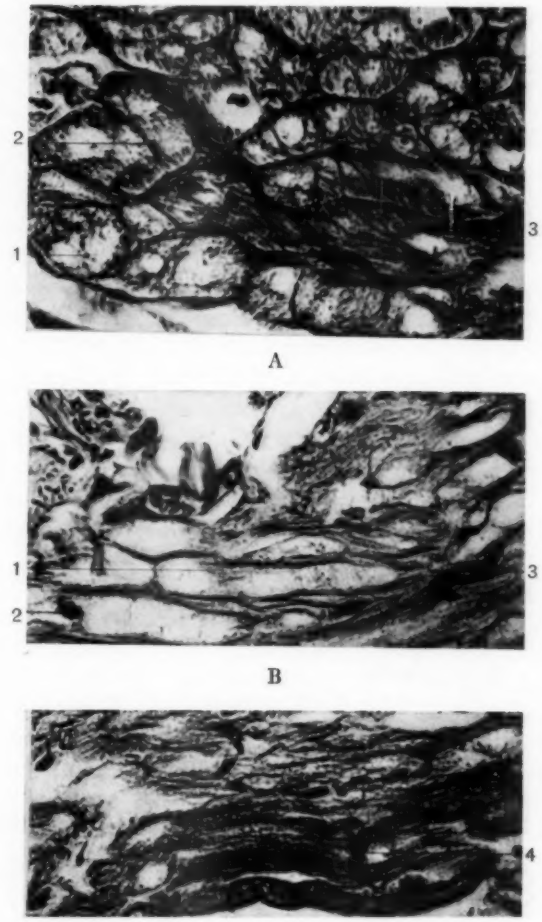


FIG. 1. MICROPHOTOGRAPH OF AURICULO-VENTRICULAR NODE OF HUMAN HEART (×350)

A. Central part of A-V node. *1.* Purkinje cell-cut in cross section. *2.* Septum partially dividing a large Purkinje cell. *3.* Specialized tissue, cut longitudinally.

B. Transitional tissue between node and heart muscle. *1.* Purkinje cell cut longitudinally. *2.* Central nucleus of a Purkinje cell. *3.* Transitional tissue.

C. Cardiac muscle shown clearly at *4.*

more marked, (fig. IB). This continues until finally the cells have all the characteristics of typical cardiac muscle, (fig. IC). Thus in certain places in the A-V node it is impossible to say where the specialized tissue ends and the cardiac muscle begins.

This fact was brought out by Tawara (46) in his original study of the A-V node. It has also been emphasized by Cohn (6) and by Pace (36). Schander (40), in his study of the A-V node in the horse, described a group of fibers lying near the coronary sinus and the inferior vena cava which "converge to form the A-V node." Therefore it is evident that our observation is neither unique nor new. However, because it is so frequently lost sight of, it seemed advisable to stress the fact that in certain places there is a gradual transition from the auricular cardiac muscle to the A-V node.

Our knowledge of the S-A node is much more limited. The problem is much more difficult because the node cannot be discerned grossly with any certainty. It is known to lie at the junction of the superior vena cava and the right auricle. The general region of the sino-auricular node is shown in Plate 1, figure 1. However, there is some difference of opinion as to its exact location. Thus the position of the sino-auricular node according to several authorities is shown in figure 2.

Keith and Flack (14) state that, "macroscopically, the fibers resemble those of the A-V node in being paler than the surrounding musculature, *i.e.*, in being of the white variety. They can be dissected out on the superior vena cava in the region corresponding to the right venous valve." Their line indicating the location of the S-A node lies at right angles to the sulcus terminalis and the maximum amount of nodal tissue lies at x, as shown in figure 2A.

In a later paper Keith and Mackenzie (15) state that "the sino-auricular nodal tissue becomes less in amount and more concentrated in position as one passes from the lower to the higher types of hearts." In the hearts of rodents the nodal tissue has an extensive distribution along the junction of the superior vena cava and the right auricle, but in man it tends to become more concentrated at the termination of the superior vena cava.

Cohn (8) and Lewis (28, 29) both locate it to the right of the sulcus terminalis, as shown in figure 2B.

The Oppenheimers (35) state that the node "lies at the junction of

the superior vena cava and the right auricle immediately on the venous side of the remnants of the old valves."

Koch (20, 21) also locates the sino-auricular node in the sulcus terminalis (24) on the outer and posterior wall of the superior vena

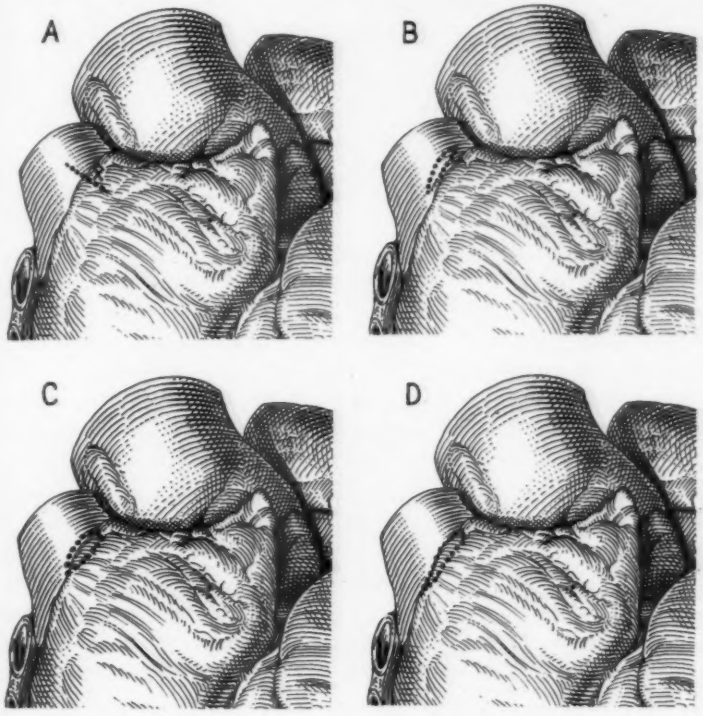


FIG. 2. DETAIL OF PLATE 1 SHOWING THE LOCATION OF THE SINO-AURICULAR NODE

A. According to Keith and Flack.
B. According to Lewis.
C. According to Koch.
D. According to Bachmann.

cava, as shown in figure 2C. Indeed he describes it as a band of special muscular tissue which encircles the superior vena cava and connects

it with the right auricle, *i.e.*, it corresponds to the sinus venosus in the embryo.

Bachmann (2), in his study of the nerves of the sino-auricular node, locates the node on the auricular side of the sulcus terminalis, as shown in figure 2D.

Therefore, a large block of tissue was taken. The upper margin of the block extended through the middle of the superior vena cava and the lower margin of the block extended, as we thought, well into the auricle. This block was so large that it was cut into four small blocks to insure proper fixations. They were fixed in Zenker's and embedded in paraffin in the usual way. Serial sections were cut perpendicular to the S-A node. This plane of sectioning was chosen because most authors[4] agree that, in order to obtain satisfactory preparations of the node, sections must be cut at right angles to the node. By so doing one cuts through the artery of the S-A node and "by its aid one can readily locate the node." This method makes the node stand out clearly as a dense compact mass of tissue surrounding the artery.

This was quite true in our sections. Throughout nearly the entire series of sections one could readily see a mass of tissue which corresponded exactly to the classical descriptions of the S-A node. It is a dense compact mass rich in connective tissue. The cells are closely interwoven into a knot or "node" and cut at all sorts of angles. Through the center of this structure runs an artery, the artery of the S-A node (11, 13, 14). A low-power photograph of the node is shown in figure 3. Little structural detail can be made out in the central portion of the node. However, scattered through this mass of connective tissue are small islands of specialized tissue, as shown in figure 4A. The cells near the margin of the node are larger than those in the center. Indeed here they are as large as the cells of the A-V node, as shown in figure 4B.

This curious mass of nodal tissue, at first glance, appears to be sharply separated from the surrounding tissue. The difference between the two is even more exaggerated when a connective-tissue stain is used, for the color which the node takes is affected by the large amount of connective tissue present.

[4] Keith and Flack, (14), Koch (21), Cohn (8), Krumbhaar (27).

FIG. 3. MICROPHOTOGRAPH OF A SECTION THROUGH THE SINO-AURICULAR NODE OF THE HUMAN HEART (×90)

Block 1 shows central part of the S-A node which is shown in high power in figure 4 a.

Block 2 shows the marginal part of the S-A node. High power microphotograph of this is shown in figure 4 b.

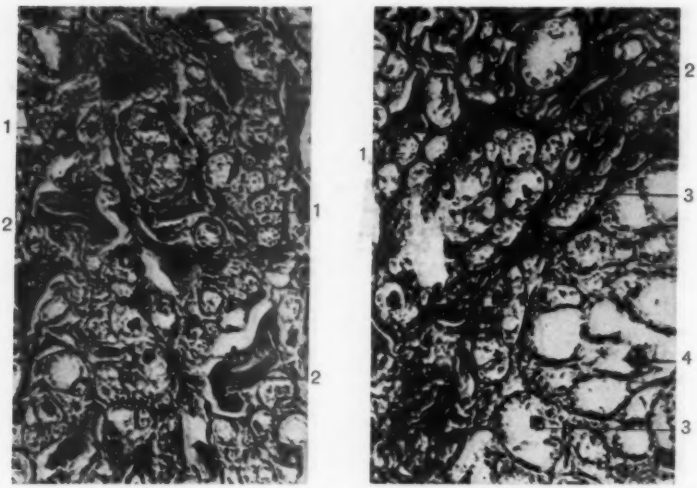
Block 3 shows location of the Purkinje cells on the Superior Vena Cava, i.e., the area from which figure 5 was taken.

Block 4 shows the region on the Superior Vena Cava from which figure 6 was taken.

Block 5 shows the area of the right auricle in which the large cells shown in figure 7 were found.

Block 6 shows the area from which figure 8 was taken showing ordinary cardiac muscle.

Photographs and drawings of the node are indeed few.[5] One of the best is that by Krumbhaar (27). Comparing our sections with his photographs, one can readily see that it is the same tissue. Indeed, the sections are very much alike. Nevertheless, they differ from his in one significant detail. Our sections were cut to include part of the



A B

FIG. 4. MICROPHOTOGRAPH OF THE SINO-AURICULAR NODE OF THE HUMAN HEART (×350)

A. Central part of the S-A node. *1.* S-A nodal cell. *2.* Connective tissue.

B. Marginal part of S-A Node. *1.* S-A nodal cell. *2.* Connective tissue. *3.* Nucleus of larger nodal cell. Cell shows clear zone about nucleus. *4.* Nucleus of a large specialized tissue cell, note the central clear zone and the septa partially dividing cell.

superior vena cava. In this bit of tissue one sees a strand of specialized tissue cut longitudinally, as shown in figure 3, block 3. Under high power these cells are seen to have relatively large central nuclei

[5] The articles by Burian (4), Cady (5), Holmes (13), Koch (23, 24) Krumbhaar (27), Lewis (28), Schönberg (41, 42), Tandler (44), Tawara (46) and Yater (53), give illustration for the S-A node.

surrounded by a zone of clear cytoplasm; at the cell borders are delicate interweaving fibrillae whose longitudinal striations are much more pronounced than the cross striation. These cells are shown in figure 5. In short, they are beautiful Purkinje cells. Furthermore, in cross section they have the characteristic vacuolated appearance with the septa partially or completely dividing the cell.

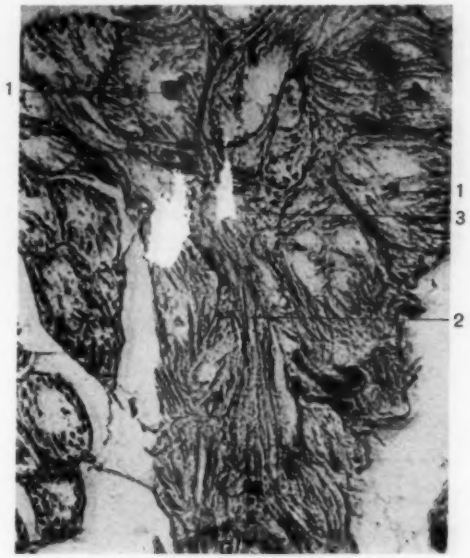


FIG. 5. MICROPHOTOGRAPH OF PURKINJE CELLS ON SUPERIOR VENA CAVA OF HUMAN HEART (×350)

1. Nucleus of a large Purkinje cell. *2.* Purkinje cell cut longitudinally. *3.* Free anastomosis of fibrillae from cell to cell. It is almost impossible to determine the cell borders in the center of the photograph.

As one follows through the sections serially, one can see these large specialized tissue cells approaching the sino-auricular. The cells themselves gradually become a little smaller and the cells at the margin of the node become larger until finally at the point where the two types of tissue meet one can hardly distinguish the one from the

other. Thus, there can be no doubt that this strand of specialized tissue emerges from the sino-auricular node. Numerous observers[6] have claimed that the sino-auricular extended up onto the superior vena cava, but the fact that the cells on the superior vena cava are

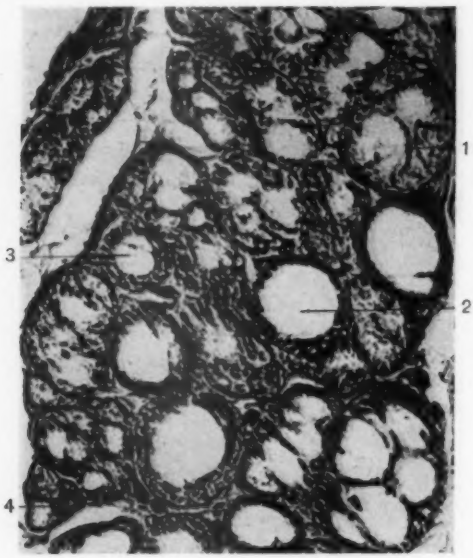


FIG. 6. MICROPHOTOGRAPH OF THE CELLS ON THE SUPERIOR VENA CAVA OF HUMAN HEART (×350)

1. Large Purkinje cells with relatively clear center, marginal fibrillae. A septum is seen partially dividing the cell. *2.* Large Purkinje cells showing clear central zone and marginal fibrillae. *3.* Cell of intermediate size with more fibrillae than cell No. *2* but is larger than auricular cardiac muscle cells and still shows clear central zone. *4.* Auricular cardiac muscle cells. Note the small size and the denser structure.

larger than the cells of the node itself has not been emphasized. Indeed they are Purkinje cells rather than nodal cells.[7]

[6] Keith and Flack (14), Koch (21, 23), and others.

[7] In this connection Rylant's (39) work is of interest. He claims that the impulse of contraction originates not at the S-A node but further back on the superior vena cava.

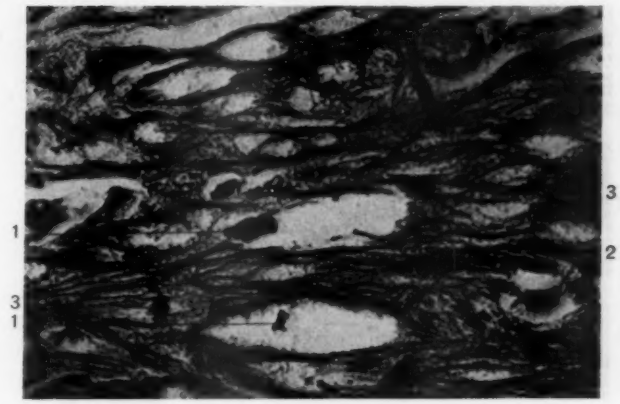


FIG. 7. MICROPHOTOGRAPH OF THE LARGE CELLS EXTENDING INTO RIGHT AURICULAR WALL OF HUMAN HEART (×350)

1. Central nuclei of the large cells surrounded by a zone of clear cytoplasm. *2.* Marginal fibrillae. *3.* Marks interweaving of the fibrillae from cell to cell.

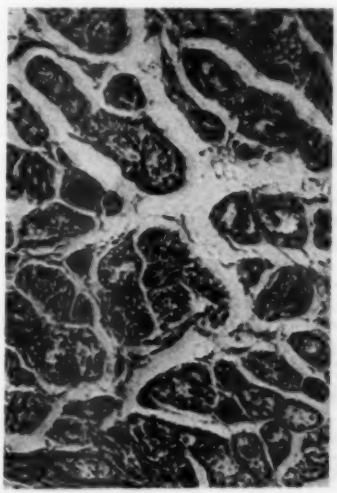


FIG. 8. MICROPHOTOGRAPH OF AURICULAR CARDIAC MUSCLE OF HUMAN HEART SEEN IN CROSS-SECTION (×350)

On either side of this strand of tissue the cells are cut in cross section. Comparing these cells (figure 6) with auricular cardiac muscle cells (figure 8) one finds these cells are much larger than the muscle cells. The central portion of the cell is clear and the cortical zone is formed by a narrow band of myofibrillae. In these cells one sees septa partially and completely dividing the cells. One can find all gradations in the size of these cells from the large ones to ordinary cardiac muscle cells, as shown in figure 6. Certainly the larger cells are much more closely related to the cells of the specialized tissue than to the ordinary heart muscle cell.

Again at the lower margin of the sino-auricular node, *i.e.*, as the node approaches the inner wall of the auricle, one sees some cells which are larger than the others, more vacuolated and which in longitudinal section show interweaving fibrillae, as shown in figure 7. Such cells are seen extending into the folds of the auricular musculature. One cannot but think that they represent speciallized tissue. How far such cells extend into the auricular wall is now under investigation.

These findings are quite different from the classical descriptions of the sino-auricular node, for the node is usually pictured as quite distinct and separate from the auricular musculature (28). Wilson (52) describes it as "a club-shaped island of special tissue," Cohn (8) describes it as "an irregular inverted cone with a long tapering apex" but he does not discuss its relation to the auricular muscle.

Keith and Flack (14) describe it as "peculiar musculature surrounding the artery of the sino-auricular junction" and in a discussion of the node in the mole heart they say "by its connections it is undoubtedly muscular." However, they give no detailed description of its boundaries or connections.

Koch (21, 23, 25) describes the node as triangular in cross section and states that the specialized tissue can be traced in the superior vena cava and into the auricle.

Pace (36), in 1924, described two separate sino-auricular nodes in the sheep heart, one lying in the sulcus terminalis and the other, the so-called left sino-auricular node, in the inter-auricular wall.

Recently Segre (43), on the basis of serial sections of four cases, two infants, one child, and one young adult, claimed that there are not two nodes, a right and a left, but one large horse-shoe-shaped node.

One end of this node is composed of the "S-A"; the other end is the left S-A node of Pace; while the loop of the horse-shoe lies posterior to the superior vena cava.

Recently Nomura and Ida (33) have reviewed the subject with special regard as to the possibility of a pathway between the sino-auricular node and the atrio-ventricular node. Such a connection was long ago claimed by Thorel (47, 48) and immediately denied by Koch (22). The Japanese authors again deny it. They claim that the cells which have been thought to be Purkinje cells are really pathological cardiac muscle cells.

Tander (44) and Todd (50) have made no special study of the sino-auricular node but both believe there is specialized tissue throughout the auricle. Tandler's conclusions are based upon extensive work on human hearts. One of his studies was made upon the heart of a criminal 28 years old. This case offered unique opportunity for the prompt fixation of a normal heart. In this heart, as well as in others, he found a considerable number of cells which resembled the cells of specialized tissue. Since there was no reason to suspect any cardiac pathology, he felt quite certain that these cells were normal Purkinje cells and not cardiac cells which had undergone pathological degeneration. Todd, as a result of his studies on the specialized tissue, believes that there is no part of the auricle devoid of Purkinje cells. Indeed he states that in his opinion the sino-auricular node and the atrio-ventricular node are merely "atrial masses of Purkinje fibers easily located and easily demonstrated."

Rothberger (37, 38), although he has not worked upon the problem, has reviewed the literature and emphasized its importance in regard to the cardiac arrhythmias.

The problem of the extension of the specialized tissue into the auricle and the possibility of the existence of a conducting system in the auricles is now under investigation.

In *summary* this study shows that in the human heart:

1. In certain places at the margin of the A-V node one can trace a gradual transition from the A-V nodal tissue to the auricular cardiac muscle.

2. The specialized tissue cells at the outer margin of the sino-auricular node are larger than those in the center of the node.

3. There are Purkinje cells extending from the S-A node into the outer wall of the superior vena cava.

4. There are cells closely resembling specialized tissue cells extending from the S-A node into the folds of the auricular musculature.

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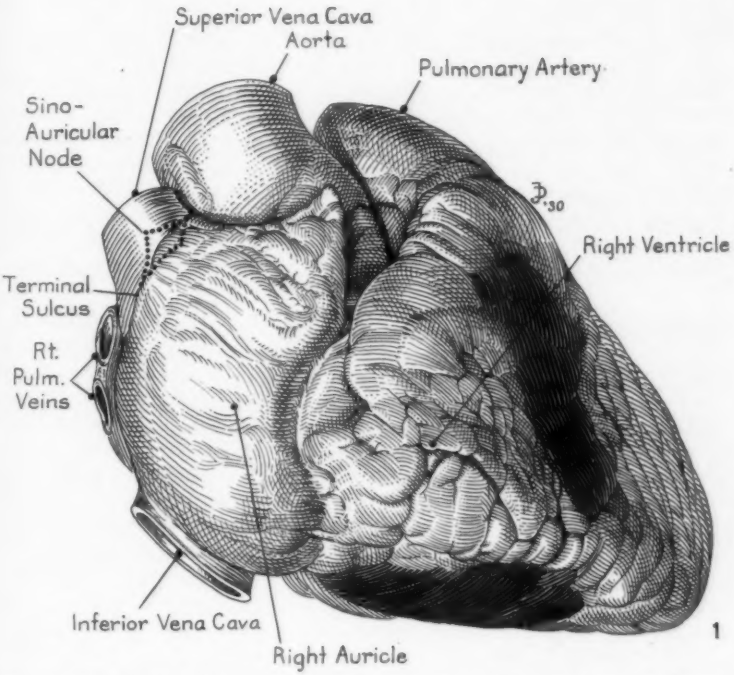

FIG. 1. DRAWING OF VENTRAL ASPECT OF THE HEART SHOWING THE GENERAL LOCATION OF THE SINO-AURICULAR NODE

BOOK REVIEWS

The Principles of Bacteriology and Immunity. By W. W. C. Topley, M.A., M.D., M.Sc., F.R.C.P. and G. S. Wilson, M.D., M.R.C.P., D.P.H. Vols. I and II. 1320 pp. $15.00. (*William Wood and Company, New York, 1929.*)

This work of 1320 pages in two volumes seems to us to be the best textbook for students that has appeared in English. It may be recommended also as a reference book which will meet most of the requirements of physicians interested in Bacteriology and Immunology. It is divided into four parts: I. General Bacteriology; II. Systematic Bacteriology; III. Infection and Resistance; IV. The Application of Bacteriology to Medicine and Hygiene. It is well arranged, well written. The material is adequate and well chosen. In most respects it is as nearly up-to-date as one should expect a textbook to be. The illustrations are not numerous but are adequate.

It is always easy for a reviewer to criticize certain details; for instance, we miss the usual chapter on media which is always a convenience for reference. There is not an adequate description of Streptococcus epidemicus or of septic sore throat and its relation to bovine mastitis. Many will question the inclusion of Bact. bronchisepticum among the Brucella and some details in the description of the Morax-Axenfeld bacillus. It is also questionable as to whether the necrophorus bacillus should be placed in the genus Fusiformis. However, it is a satisfaction to find these organisms and also Cornebacterium pyogenes and the anaerobic streptococci described or mentioned. The cornebacteria are treated with more detail than is customary in a student textbook. There are excellent bibliographies at the end of each chapter.

J. H. B.

Materia Medica and Therapeutics, Including Pharmacy and Pharmacology. By R. W. Wilcox. Twelfth Edition. (*P. Blakiston's Son & Co. Inc., Philadelphia, 1929.*)

The book is divided into two parts. Part I deals with "Materia Medica and Pharmacy." Part II considers "Pharmacology and Therapeutics." Part I is rather satisfying in its brief and adequate presentation of a number of useful pharmaceuticals. In it however one is rather surprised to find that the dose of arsphenamine is "0.4 Gm. = 60 milligm." while that of neoarsphenamine is "0.6 Gm. = 60 milligm." and that of the tincture of digitalis is 0.1 cc. or 15 m. These are gross errors but they could have been pardoned had your reviewer not gone on to read Part II.

In the latter section one finds that the space devoted to the therapeutics of *Arsphenamine* is approximately the same as that devoted to the therapeutics of

Pomegranate and is considerably less than that devoted to Mustard. "Certain synthetic compounds of arsenic, Arsphenamine in dose of 0.4 Gm. (6⅔ gr.) dissolved in 300 cc. (10 oz.) of normal saline solution to which about 23 drops of a 15 per cent solution of sodium hydroxide have been added, and neoarsphenamine, mixed with one-half of its weight of inert substance, in dose of 0.6 Gm. (9⅞ gr.), have yielded brilliant results in syphilis, both, by intravenous injection as shown by the disappearance of the spirochetes from the blood." These are his complete directions for the administration of Arsphenamine; no mention of the fact that the injected product *must* be alkaline; no caution about the clarity or turbidity of the solution. He speaks of a "number of fatal results" of therapy, but gives no hint of the various Arsphenamine reactions or their treatment.

Concerning *digitalis:* "Since the infusion represents the entire drug, this is the preparation of choice." In using digitalis "it is advisable to begin with small doses." After calling attention to the present day "administration of massive doses," he says: "Some have even advocated its administration in dosage in accordance with the weight of the patient, whereas the careful practitioner knows that the physical condition of the heart is the determining factor." "It should be continued no longer than is necessary to re-establish compensation." In discussing the indications for its use in heart disease (which is only one of the many diseases in which he advises its utilization), we learn among other things that "there can be little question that in *uncomplicated aortic regurgitation the drug is injurious.*" In the light of our best information I find it impossible to accept any of these statements. For most purposes the infusion is *not* "the preparation of choice;" it is rarely "advisable to begin with small doses," and it is frequently advisable to continue its use "longer than is necessary to re-establish compensation;" animal and clinical experimentation have shown that the body-weight method is probably the most rational method of dosage; and there is considerably more than a "little question" about the "injurious" effect of the drug in aortic regurgitation.

Concerning the *antitoxic sera*, of which he mentions two, the information is too inadequate to be safe. Nothing is said of the dangers attending their usage in patients with allergic disease such as asthma, and there is no discussion of cutaneous tests or methods of desensitization. He gives no description of the *anaphylactic* serum reaction and says not a word about the necessity for having adrenalin at hand to avoid serious consequences. Ignoring these important points he invites trouble by saying that diphtheria antitoxin has been "employed with some success in the treatment of asthma."

Of *carbon tetrachloride* in the treatment of hook-worm disease, he says, "It sometimes occasions headache and nausea and also drowsiness, but there have been no known fatalities." [For fatalities see Lambert (1923), Phelps & Hu (1924), and others.]

In brief, your reviewer finds that this text contains little to recommend it to any class of readers. The consideration of the minor drugs and the treatment of the minor ailments are the high lights of the book. I am particularly grateful to him for

one sentence: "A round mass of metallic antimony (not official) was formerly known as the 'family pill,' because it could be repeatedly used as a laxative." Taken all in all, however, too much space is given over to these minor drugs and too little is devoted to those of major importance. In the excerpts above I have attempted to display examples of the reading matter upon which this impression is based. Digitalis, arsphenamine, antitoxins, carbon tetrachloride, and other drugs are therapeutic materials which find wide application and should be used intelligently, with every consideration of the best available opinions. They can hardly be used with success or safety by one who follows too implicitly the directions set forth in this volume.

J. B. III

Prescribing Occupational Therapy. By WILLIAM RUSH DUNTON, JR. 142 pp. $2.10. (*Charles C. Thomas, Baltimore, 1928.*)

"Prescribing Occupational Therapy," by William Rush Dunton, Jr., is written with an aim to stimulate the interest of the busy physician and medical student to whom this branch of therapy is little known. The author includes the special application of prescribed occupation not only for the treatment of mental cases, but also for medical, surgical, orthopedic, cardiac and tuberculous patients, as well as invalid children. There is a very helpful discussion of the varieties of work that can be adapted to the various types of patients, with special emphasis on the avoidance of fatigue and the stimulation of interest resulting in a better morale, as well as in a definite physical improvement which can be more or less specific, depending upon the result desired.

The author is one of the pioneers in occupational therapy for mental patients and greater detail of his personal experience would be of interest; but he includes a bibliography at the end of each chapter for the benefit of those who desire further information. The book is valuable as a reference and a general guide to occupational therapy, which is becoming recognized more and more as a necessary therapeutic aid in every hospital.

R. E. F.

Gynecology. By LYN LYLE FULKERSON, A.B., M.D., F.A.C.S. 842 pp. $9.00 (*P. Blakiston's Son and Company, Philadelphia, 1929.*)

This is a one volume textbook in which the author presents the essentials of medical and surgical gynecology. He has accomplished his purpose admirably, and has given us an excellent textbook for students, a good reference book for practitioners and a section on operative technique that may be read with profit by the abdominal surgeon. The author defines gynecology as "that branch of medicine which treats of woman's constitution and diseases, especially the genital, urinary and rectal diseases." He then outlines his text in accordance with the idea expressed in this definition. The value of a knowledge of urology to the gynecologist can scarcely be questioned by any one familiar with gynecological symptomatology.

The author illustrates this point very strongly in stating that a clinical study made in the Gynecological Department of Cornell University Medical School revealed that 28.7 per cent of the 1039 women examined complained of urinary symptoms.

The chapter on anatomy is the usual one and is illustrated by many of the best anatomical illustrations chosen from various standard textbooks of anatomy and gynecology. The physiology of the female generative organs is discussed in its relation to the other internal secretory glands. The, at present, accepted view of the physiology of menstruation is described and the histological cycle of the endometrium is particularly well illustrated.

The chapter on normal and abnormal forms and positions of the uterus is, on the whole, well written and well illustrated. In one respect, however, it would seem to be somewhat deficient. One gains the impression that the mere presence of a retroverted uterus calls for correction either by operation or pessary. In the opinion of the reviewer, the most important point to be taught medical students about this condition is the criteria upon which the decision is made as to whether the retroverted uterus requires treatment or may be allowed to remain untreated. Fulkerson makes no mention of the fact that the majority of retroverted uteri need not be corrected.

The weakest point in the volume is the description of the pathology of the endometrium in the chapter on the diseases of the uterus. In discussing chronic endometritis the author states it "may be a hyperplasia due to displacement of the uterus or chronic adnexal disease rather than a true inflammation." The use of the term endometritis to designate any condition but a true inflammation is certainly not advisable. It is true the author has a great deal of precedent for such a statement but why continue to confuse the minds of medical students with loose pathological terms? He continues in the next paragraph:– "The gland changes may, at times, become exaggerated to form an overgrowth of lymphoid tissue." To the reviewer this statement conveys no meaning. It is not good pathology to speak of glandular epithelium forming lymphoid tissue.

The author's definition of hyperplasia of the endometrium as "a thickening of the mucous membrane beyond the normal that occurs in the phases of menstruation" is hardly the correct definition of the condition as understood today. Microscopic hyperplasia of the endometrium is often found in endometrium which is not grossly thickened.

The chapter on diseases of the urinary tract covers the diseases of the female urinary tract in a clear, concise way and is admirably suited to the needs of the medical student. The same can be said concerning the chapter on the diseases of the anus and rectum.

The more recent advances in gynecology are discussed briefly, but clearly, in chapters on iodized oil in gynecology, radium, electrothermy, the sedimentation test, etc. Each subject is brought up to date and a fair estimate of its value and indications is given.

The high light of the book is found in the description of the various gynecological

operations. From the multitude of operative procedures described in the literature the author has shown wisdom in his selection of those best adapted to meet the various conditions. The vaginal plastic operative procedures shown are particularly satisfactory. The text describes them clearly and the drawings illustrate them well.

Taken as a whole, I feel that the book can be recommended to medical students as a very satisfactory and well balanced textbook.

R. W. TeL.

Overcoming Tuberculosis, an Almanac of Recovery. By GERALD B. WEBB and CHARLES T. RYDER. 3rd ed. revised. 81 pp. $2.00. (*Paul B. Hoeber, Inc., New York, 1927.*)

Overcoming Tuberculosis, An Almanac Of Recovery, is an elaboration of the "Recovery Record" by the same authors and is, in fact, the third edition of that book.

Primarily, the purpose of the Almanac is to encourage the tuberculous patient to chart day by day a record of "the temperature, pulse and certain other features of the weather prevailing in this body" as an essential in the control of his treatment.

The charts are ruled so that not only the temperature and pulse may be recorded, but there is also space to enter notes and questions to be asked the physician. Each page has interesting quotations from the most diversified sources and these are to help pleasantly and profitably to occupy the recorder, and to memorize some and to seach out their origin is recommended as a non-fatiguing endeavor.

The text of the volume precedes the section of charts and the succinct, lucid way in which rules of living and regulations of all activities are given him who would seek successfully to recover from pulmonary tuberculosis is admirable. The admonition and counsel, given usually in prosaic and didactic fashion, are presented here so charmingly that they are likely to encourage cheerful following of wise direction.

In placing this book in the hands of a tuberculous patient, one will install an active ally in the battle against the disease.

C. R. A.

Dermatology and Syphilology for Nurses Including Social Hygiene. By DR. JOHN H. STOKES. 311 pp. With 69 Illustrations. (*W. B. Saunders Company, Philadelphia, 1930.*)

This book carries out well the intention of the author to furnish a text for the nurse in a hospital clinic, the nurse assisting in the private office of a physician, and the nurse in the field, which will be satisfactory to all three. It gives concise but excellent descriptions of the chief diseases of the skin and the manifestations of syphilis and gonorrhoea, followed by the methods of treatment with the technic of their preparation and of nursing care which are most universally approved.

The first two parts deal with the main dermatological diseases grouped according

to the type of their lesion and similarity of treatment. The third part, on syphilis, is very thorough, from the general discussions of the social aspects to the specific directions for preparing intravenous solutions. The fourth part is concerned with the principles of social hygiene and presents many of the problems of venereal infections in a plain and thought-arousing manner which is highly commendable.

F. R. S., Jr.

Consecratio Medici and Other Papers. By Harvey Cushing, M.D., Surgeon in Chief of the Peter Bent Brigham Hospital, Professor of Surgery in Harvard Medical School. 276 pp. $2.50. *An Atlantic Monthly Press Publication. (Little, Brown, and Company, Boston, 1928.)*

This delightful volume of 276 pages contains fourteen collected addresses by this well-known surgeon and author. They have all been previously published. The volume derives its name from the first address of the series, which was delivered before the Graduating Class at Jefferson Medical College, Philadelphia, June 5, 1926. While most of the addresses were delivered in recent years, the second one entitled Dr. Garth, the Kit-Kat Poet, was read before the Johns Hopkins Hospital Historical Club, as far back as December 12, 1904.

In order that the prospective reader may get a fair idea of the subjects dealt with, the titles of the remaining twelve addresses are, in order, as follows: Realignments in Greater Medicine; With The British Medical Corps In France; William Osler, The Man; The Personality Of A Hospital; The Physician And The Surgeon; Louisa Parsons And Her Medals; The "Boston Tins;" The Clinical Teacher And The Medical Curriculum; The Western Reserve And Its Medical Traditions; Experimentum Periculosum: Judicium Difficile; The Doctor And His Books; Emancipators.

Those who have read Harvey Cushing's two volumes on the Life of Sir William Osler, for which he received the Pulitzer Literary Prize, can judge the delight in store for them in reading these delightful essays. Few, if any other, in our profession of to-day possess the medical historical back-ground and charm of expression shown in this collection of addresses. The reading of them should broaden the medical outlook of the members of our profession, both physicians and medical students.

Manual of Physiology for Students and Practitioners. By H. Willoughby Lyle, M.D., B.S. (Lond.), F.R.C.S., and David de Souza, M.D., B.S. (Lond.), F.R. C.P. (Lond.). 3rd ed. 8°. $5.25. *(Oxford, Oxford University Press, 1930.)*

This new edition of Lyle and De Souza's Manual presents physiology in a concise and definite manner, especially useful for the student who likes thoroughly predigested knowledge for beginning his work. A large amount of material contained in this book might well have been omitted, such as the chemistry of pro-

teins, feeding of infants, stages of labour, in view of the fact that the student obtains them in other courses.

C. L. G.

A Text-Book of Physiology. By WILLIAM H. HOWELL, Professor of Physiology in the School of Hygiene and Public Health, Johns Hopkins University. 11th edition. Thoroughly revised. 1099 pp. 8°. $6.50. (*Philadelphia*, W. *B. Saunders Co., 1930.*)

The difficulties confronting an author of a modern text-book in medical sciences are clearly stated by Dr. Howell in the preface to this eleventh edition. With the increasing number of articles published on physiological subjects and with the increase in specialization in the various fields of physiology, the preparation of a text-book covering completely the whole field of physiology is practically impossible. If physiology was based on a few fundamental principles and less given to controversial theories, the writing of a text-book in physiology would be as easy as the writing of an elementary text in chemistry or physics. Thus, one of the major difficulties is in deciding how far these theories may be used in showing the modern trend of the subject.

Nevertheless, Dr. Howell in a conservative manner has been able to keep his text-book abreast of the rapid growth of physiology in the fundamental principles. In this edition new material has been inserted or the former paragraphs have been changed on the newer knowledge of the phosphoric acid compounds in muscular contraction, cardiac output, function of the gall-bladder, bactericidal properties of the skin, physiological action of glands of internal secretion, intermediate metabolism of foodstuffs and physiological oxidation. The majority of the illustrations in the former edition have been retained; a few changed, such as the insertion of a more recent form of thread galvanometer. Most of the newer references are to review articles in which the student will be able to obtain a full discussion of any particular topic. Otherwise the book is practically the same as the former edition.

From the students' viewpoint, Dr. Howell's book still holds the high position it has always held among texts of physiology.

C. L. G.

The Action of Muscles, Including Muscle Rest and Muscle Re-education. By SIR COLIN MACKENZIE, M.D., F.R.C.S., F.R.S. (Edin.), Professor of Comparative Anatomy and Director of the Australian Institute of Anatomy, *etc. etc.* 2nd Edition. xvi, 288 pp. 8°. $3.50. (*New York, Paul B. Hoeber*, 1930.)

Man has long been interested in the action of his muscles. The first thorough study of muscular action was that of Aristotle, the results being contained in his two books, "*De Incessu Animalium*" and "*De motu animalium.*" Leonardo's *Quaderni* contain many interesting sketches of muscular action with mechanical analyses. Borelli's classic "*De motu animalium*" (1681) was the outstanding

production of the Iatro-Mathematical School. In more modern times, the Great War served to focus attention on muscular action. Sir W. Mackenzie states that 65 per cent of wounded men, returned from the battlefields, were suffering from disabilities in which the question of muscular function became of prime importance with reference to treatment.

In the first chapter of this book, the author discusses the principles of muscular action, anatomical relationships of muscle, ligaments and bone, pulley and lever action of muscle, muscular coordination, advantages of the erect posture and other fundamental themes.

The succeeding chapters deal with muscular action from the normal and abnormal aspect. Each separate anatomical region is discussed from the standpoint of single muscular action as well as the action of groups of muscles. Anatomical deformities resulting from nerve paralysis, position of rest, methods of reëducation with clinical applications are treated in detail, with many clear photographs and diagrams. References to comparative physiology also illustrate particular points.

There are a few discordant notes. The author attempts to show a relationship between the thymus and muscular coordination. He suggests that since the thymus in man is at its maximum development when muscular action and coordination associated with erect posture are developing, and begins to dwindle when coordination is well established, the thymus has something to do with this development of muscular coordination—a wholly unwarrantable assumption. He even suggests feeding thymus gland to patients in the early stages of reëducation of muscles following poliomyelitis. He goes still further afield and hypothecates that the thymus gland in man may in some way be associated with failure of regeneration of a limb after amputation.

Again, the author states that, following Newton's discoveries, the nerves and muscles were regarded as containing a "rare elastic spirit." This doctrine goes back to Galen, whose "animal spirits" were just as rare and elastic as those of Newton's time.

This book commends itself to students of anatomy as clearly showing that the best way to study muscular function is in the living, not in the dissecting room. To the physiologist, whose interest during the past twenty years has been confined mainly to the mechanism inside the muscular fiber, it recalls the fact that the prime interest of muscle is that it exists for moving bones, and not as a mechanical structure that in an isolated state adapts itself readily for experimentation. To the clinician, the book gives a rational anatomical background for his clinical findings and lines of treatment in muscular deformities.

The present edition is an enlargement on the first, a special section having been added dealing with the erect posture.

C. L. G.

BOOKS RECEIVED

A Text-book of Histology. Arranged upon an Embryological Basis. By J. Lewis Bremer, M.D. Fourth edition of "Lewis and Stöhr." Four hundred and eighty-six illustrations thirty-two of which are in color. 1930. 8°. 568 pages. P. Blakiston's Son and Company, Inc., Philadelphia. $6.00.

Alimentary Anaphylaxis. (Gastro-intestinal Food Allergy). By Guy LaRoche, Charles Richet Fils and François Saint-Girons. Foreword by Prof. Charles Richet. Translated by Mildred P. Rowe and Albert H. Rowe. 1930. 12°. 139 pages. University of California Press, Berkeley. $2.00.

Massage et Rééducation. Par Le Dr. Durey et Le Dr. Lucienne Meurs. Les Actualités Physiothérapiques. V. Kinésithérapie. 1930. 12°. 95 pages. Gauthier-Villars et Cie, Paris. Frs. 15.

Problems and Methods of Research in Protozoölogy. Edited by Robert Hegner and Justin Andrews. 1930. 8°. 532 pages. The MacMillan Company, New York. $5.00.

A Textbook for Massage for Nurses and Beginners. By Maude Rawlins. 1930. 12°. 1444 pages. The C. V. Mosby Company, St. Louis. $2.00.

Medicine in the British Isles. By Sir D'Arcy Power, K.B.E., F.R.C.S. (Eng.). Clio Medica II. 1930. 16°. 84 pages. Paul B. Hoeber, Inc., New York. $1.50.

Physical Diagnosis. By Richard C. Cabot, M.D. Tenth edition. Revised and enlarged, with six plates and 279 figures in the text. 1930. 8°. 529 pages. William Wood and Company, New York. $5.00.

Personal and Community Health. By Clair Elsmere Turner, M.A., Dr.P.H. Third edition. 1930. 8°. 443 pages. The C. V. Mosby Company, St. Louis. $2.75.

Pernicious Anaemia. By Leybourne Stanley Patrick Davidson, B. A. (Camb.), M.D., F.R.C.P.E., and George Lovell Gulland, C.M.G., LL.D., M.D., F.R.C.P.E. With Appendix on Dietetic Treatment by Ruth Pybus. With Introduction by Lawrence D. Thompson, M.D. With 8 illustrations and 22 plates of which 12 are in colour. 1930. 8°. 293 pages. The C. V. Mosby Company, St. Louis. $8.50.

Dietetics and Nutrition. By Maude A. Perry, B.S. 1930. 12°. 332 pages. The C. V. Mosby Company, St. Louis, $2.50.

Gonococcal Infection in the Male. By Abr. L. Wolbarst, M.D. Second edition, completely revised and enlarged. With 140 illustrations, including 7 color plates. 1930. 8°. 297 pages. The C. V. Mosby Company, St. Louis. $5.50.

Doctor and Patient. Papers on the Relationship of the Physician to Men and Institutions. By Francis Weld Peabody, M.D. 1930. 12°. 95 pages. The MacMillan Company, New York. $1.50.

Oxidation-Reduction Potentials. By L. Michaelis, M.D. Translated from the German Manuscript by Louis B. Flexner. 16 illustrations. 1930. 8°. 197 pages. J. B. Lippincott Company, Philadelphia. $3.00.

A System of Bacteriology in Relation to Medicine. By F. E. ANDREWES, J. A. ARKWRIGHT, J. E. BARNARD and Others. Volume 1. 1930. 8°. 374 pages. (Medical Research Council). His Majesty's Stationery Office, London.

Oxford Medical Pulications. Humphrey Milford Publisher, London. The following nine volumes.

Procedure in Examination of the Lungs. With Especial Reference to the Diagnosis of Tuberculosis. By ARTHUR F. KRAETZER, M.D. With a Foreword by James Alexander Miller, M.D. 1930. 8°. 125 pages.

A Text-Book for Midwives. By JOHN S. FAIRBAIRN, M.A., B.M., B.Ch. (Oxon.), F.R.C.P. (Lond.) F.W.C.S. (Eng.) Mast. Midw. Soc. Apoth. (Lond.). Fifth edition. Illustrated. 1930. 8°. 369 pages.

A Text-Book of Psychiatry. By D. K. HENDERSON, M.D. (Edin.), F.R.F.P.S. (Glas.) and R. D. GILLESPIE, M.D. (Glas.), M.R.C.P., D.P.M. (Lond.). Second edition. 1930. 8°. 526 pages.

Manual of Physiology. By H. WILLOUGHBY LYLE, M.D., B.S. (Lond.), F.R.C.S. (Eng.), and DAVID DE SOUZA, M.D., D.Sc. (Lond.), F.R.C.P. (Lond.). With 3 plates and 138 figures in the text. 1930. 820 pages.

Handbook of the Vaccine Treatment of Chronic Rheumatic Diseases. By H. WARREN CROWE, D.M., B.Ch. (Oxon), M.R.C.S., L.R.C.P. 1930. 12°. 52 pages.

The Physiology of the Vestibular Apparatus. By MARIO CAMIS, Director of the Institute of Physiology of the Royal University of Parma. Translated and Annotated by R. S. Creed, B.M., B.Ch., M.A. 1930. 8°. 310 pages.

Injuries to Joints. By SIR ROBERT JONES, Bart., K.B.E., C.B. Third edition. 1930. 16°. 195 pages.

Dosage Tables for Röntgen Therapy. By PROFESSOR FRIEDRICH VOLTZ. Translated from the second German edition. 1930. 12°. 120 pages. $1.50.

Aphasia in Children. By ALEX. W. G. EWING, M.A., Ph.D. With an Introduction by E. D. Adrian, M.D., F.R.C.P., F.R.S. 1930. 8°. 152 pages. $3.50.

Collected Papers on the Mayo Clinic and the Mayo Foundation. Edited by Mrs. M. H. Mellish, Richard M. Hewitt, B.A., M.A., M.D. and Mildred A. Felker, B.S. Volume XXI, 1929, published May 1930. 8°. 1197 pages. W. B. Saunders Company, Philadelphia. $13.00.

Surgical Diagnosis. By American Authors. Edited by Evarts Ambrose Graham, A.B., M.D. Containing 1250 illustrations. Volume III. 1930. 8°. 1044 pages. W. B. Saunders Company, Philadelphia. $35.00 a set.

General Index to Volumes I to III of Graham's Surgical Diagnosis. 1930. 8°. 155 pages. W. B. Saunders Company, Philadelphia.

A Textbook on the Nursing and Diseases of Sick Children. For Nurses and Welfare Workers. By Various Authors. Edited by Alan Moncrieff, M.D., B.S., M.R.C.P. Lond. With Introduction by George R. Irving, M.D. Illustrated. 1930. 8°. 580 pages. G. P. Putnam's Sons, New York. $4.50.

An Introduction to Vertebrate Embryology. By H. L. WIEMAN. First edition. 1930. 8°. 411 pages. McGraw-Hill Book Company, Inc., New York. $4.00.

Diseases of the Urinary Tract in Children. By EDWIN BEER, M.D., and ABRAHAM HYMAN, M.D. With 50 illustrations. 1930. 318 pages. Paul B. Hoeber, Inc., New York. $6.00.

Allergic Diseases. Their Diagnosis and Treatment. By RAY M. BALYEAT, M.A., M.D., F.A.C.P. Illustrated. Third edition, revised and enlarged. 1930. 8°. 395 pages. F. A. Davis Company, Philadelphia. $5.00.

The Beginnings: Egypt and Assyria. By WARREN R. DAWSON, F.R.S.E. 1930. 16°. 80 pages. Paul B. Hoeber, Inc., New York. $1.50.

La Radiothérapie Pénétrante. Par MM. LEBOUX-LEBARD et ETIENNE PIOT. Les Actualités Physiothérapiques. II. Radiothérapie, Volume 3. 1930. 8°. 80 pages. Gauthier-Villars et Cie., Paris. 15 fr.

Slit-Lamp Microscopy of the Living Eye. By Dr. F. ED. KOBY (Basle). Translated by Charles Goulden, O.B.E., F.R.C.S. and Clara Lomas Harris, M.B. Second edition. Illustrated. 1930. 8°. 360 pages. P. Blakiston's Son and Company, Philadelphia. $4.50.

Manual of the Diseases of the Eye. By CHARLES H. MAY, M.D. Thirteenth edition, revised. With 374 original illustrations including 23 plates, with 73 colored figures. 1930. 12°. 461 pages. William Wood and Company, New York. $4.00.

Sensation and the Sensory Pathway. By JOHN S. B. STOPFORD, M.D., F.R.S. With illustrations. 1930. 8°. 148 pages. Longmans, Green and Company, New York. $3.00.

Anatomy. By GEORGE W. CORNER, M.D. Illustrated. Clio Medica III. 1930. 16°. 82 pages. Paul B. Hoeber, Inc., New York. $1.50.

Allergie des Lebensalters. VON DR. CLEMENS PIRQUET. Mit. 142 Abbildungen und 1 Tafel. 1930. 4°. 170 Seiten. Georg Thieme, Verlag., Leipzig. Rmk. 25.

Recent Advances in Chemotherapy. By G. M. FINDLAY, O.B.E., M.D., D.Sc. With a Foreword by C. M. Wenyon, C.M.G., C.B.E., M.B., B.S., B.Sc., F.R.S. With 4 plates and 11 text figures. 1930. 12°. 532 pages. P. Blakiston's Son and Company, Inc., Philadelphia.